Connecting the dots
Dietz, S.M.

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Connecting the dots

Monitoring strategies and long-term consequences of Kawasaki disease

Sanne Dietz
Connecting the dots

Monitoring strategies and long-term consequences of Kawasaki disease

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Connecting the dots
Monitoring strategies and long-term consequences of Kawasaki disease

ACADEMISCH PROEFSCHRIFT

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Sanne Marieke Dietz
geboren te Amsterdam

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Contents

INTRODUCTION 7
Chapter 1 Outline of the thesis 9
Chapter 2 Dissecting Kawasaki disease – a state-of-the-art review 15

PART I – CARDIOVASCULAR RISK AND THE FOLLOW-UP OF PATIENTS AFTER KAWASAKI DISEASE 39
Chapter 3 Peripheral endothelial (dys)function, arterial stiffness and carotid intima-media thickness in patients after Kawasaki disease: a systematic review and meta-analyses 41
Chapter 4 Carotid intima-media thickness in patients with a history of Kawasaki disease 71
Chapter 5 Extracardial vasculopathy after Kawasaki disease: a long-term follow-up study 87
Chapter 6 Cardiovascular imaging techniques in children and adults following Kawasaki disease 107

PART II – GIANT ANEURYSMS 125
Chapter 7 Giant aneurysms: a gender-specific complication of Kawasaki disease? 127
Chapter 8 Regression and complications of Kawasaki disease-related giant aneurysms 147

PART III – IMMUNITY IN KAWASAKI DISEASE 161
Chapter 9 Performance of MRP8/14 and human neutrophil elastase to discriminate acute inflammatory disease in Kawasaki disease from invasive infection in childhood 163

PART IV – SUMMARY AND DISCUSSION 177
Chapter 10 Summary, general discussion and future perspectives 179
INTRODUCTION

APPENDICES 193
Dutch summary/Nederlandse samenvatting 195
Contributing authors 199
List of publications 202
PHD Portfolio 204
Word of thanks 208
About the author 210
1

Outline of the thesis
Kawasaki disease is a paediatric vasculitis mainly occurring in young children. It is characterised by prolonged fever, rash, conjunctival injection, a lymphadenopathy, and abnormalities of mucosae and extremities. The disease is self-limiting, yet complications do occur, with the development of coronary artery aneurysms (CAA) as the most important one. The percentage of patients developing CAA has dropped significantly since the introduction of intravenous immunoglobulins (IVIG) as effective treatment. Even though numerous aspects of the disease have been unravelled in the past years, many aspects including the aetiology, the susceptibility, the cardiovascular consequences and diagnostic markers remain unclear. Several of these subjects are being addressed in the studies of this thesis.

The introduction section of this thesis provides a general overview of Kawasaki disease (KD). In a narrative review, the different aspects of KD are discussed including aetiology, treatment and cardiovascular consequences. Some of the well-known as well as the more recent advances in research are being discussed (Chapter 2).

In Part I, cardiovascular risk as well as cardiovascular follow-up of children after KD is outlined. It is known that patients with CAA have an increased cardiovascular risk due to the possibility of thrombosis within or stenosis proximal or distal of a CAA. Nevertheless, concerns have been raised regarding the possibility of an increased cardiovascular risk, independent of the presence or absence of CAA, due to the systemic vasculitis. Since the majority of patients with KD are not old enough to investigate the natural course of the (cardiovascular) disease, one needs to investigate surrogate markers for cardiovascular disease risk. First, we performed a systematic review and meta-analyses evaluating surrogate markers for cardiovascular risk in patients after KD (Chapter 3). We evaluated studies looking at carotid intima-media thickness (cIMT), flow-mediated dilation, stiffness index, peripheral arterial tonometry and pulse wave velocity. We then set up a cross sectional study assessing cIMT in our own cohort of KD children (Chapter 4). We compared these children to their healthy siblings. Furthermore, since many patients had obtained multiple cIMT measurements, we also evaluated cIMT in a longitudinal manner (Chapter 5). In this study, we divided the patients after KD in different groups according to the presence or absence to CAA. As the group of healthy siblings comprised of a heterogeneous group when regarding age, we could compare the Kawasaki groups and the control group longitudinally. The (cardiovascular) follow-up of patients after KD has been a subject of debate. In Chapter 6 we describe the existing guidelines and propose pathway to follow-up these patients.

CAA can be classified according to their size, with giant CAA being at the end of the spectrum. In Part II, we further investigated the group of patient who developed giant CAA. Up-to-date, it is still unknown why some children develop (giant) CAA and other children do not. Once there is more knowledge on the risk factors for giant CAA, it may
be possible to treat these children differently. Also, there is still a lot of uncertainty about the consequences and prognosis of these giant CAA. Although some risk factors for the development of CAA have been identified, risk factors for giant CAA in Western children are undetermined. First, we aimed to find risk factors for the development of giant CAA (Chapter 7). We evaluated a group of KD patients who all had their cardiac follow-up from the acute phase onwards at the AMC. We thus created a homogenous group in which we compared the group who developed giant CAA with the group who had no enlargement, who had small CAA and who developed medium CAA. We compared these groups on gender, IVIG treatment, second IVIG treatment, age during the acute disease and presentation of disease. Additionally, we described all children with giant CAA based on z-scores; lumen diameters of the coronary arteries adjusted for basal-surface-area, who visited our multidisciplinary outpatient clinic between 1999 and 2015 (Chapter 8). We evaluated the rate of regression of these CAA as well as the occurrence of major adverse events (cardiac events or cardiac interventions) in this group.

In Part III, we evaluated inflammatory parameters during KD (Chapter 9). At the moment, there is no biomarker specific for KD. KD patients are still being misdiagnosed and thus missed, which can lead to the lack of treatment with IVIG. Finding a biomarker, specific for KD can improve the diagnosis. We measured MRP 8/14 and elastase in plasma of patients with acute KD. We compared the concentrations before and after treatment as well against patients with infectious disease such as sepsis and pneumonia.

A summary and general discussion of our findings can be found in Part IV (Chapter 10). This part provides a critical note on the results of the studies as well as perspective for future research in different research lines concerning Kawasaki disease.
Dissecting Kawasaki disease – a state-of-the-art review


Submitted
Chapter 2  Dissecting Kawasaki disease – a state-of-the-art review

Kawasaki disease (KD) is a pediatric vasculitis with coronary artery aneurysms (CAA) as its main complication. The diagnosis is based on the presence of persistent fever and clinical features including exanthema, lymphadenopathy, conjunctival injection, and changes to the mucosae and extremities. Although the etiology remains unknown, the current consensus is that it is likely caused by an (infectious) trigger initiating an abnormal immune response in genetically predisposed children. Treatment consists of high dose intravenous immunoglobulin (IVIG) and is directed at preventing the development of CAA. Unfortunately, 10-20% of all patients fail to respond to IVIG and these children need additional anti-inflammatory treatment.

Coronary artery lesions are diagnosed by echocardiography in the acute and sub-acute phases. Both absolute arterial diameters and z-scores, adjusted for height and weight are used as criteria for CAA. Close monitoring of CAA is important as ischemic symptoms or myocardial infarction due to thrombosis or stenosis can occur. These complications are most likely to arise in the largest, so-called giant CAA. Apart from the presence of CAA, it is unclear whether KD causes an increased cardiovascular risk due to the vasculitis itself. Since patients are entering adulthood, long-term follow-up is increasingly important.

Introduction

Mucocutaneous lymph node syndrome is an acute vasculitis first described by Dr. Tomisaku Kawasaki in 1967. This condition, now known as ‘Kawasaki disease (KD)’, is increasingly recognized in Western countries but has a greatly increased incidence in Japan and Asia. The main complication is coronary artery damage or coronary artery aneurysms (CAA) and KD is the leading cause of pediatric acquired heart disease in asset-rich countries.

Epidemiology

Kawasaki disease is commonest in infants and young children. The incidence varies markedly between ethnic groups. The incidence of KD in recent European studies is 5-10 per 100,000 children under the age of 5 years. A considerably higher incidence is reported in Asian countries. The highest incidence is found in Japan; the most recent nationwide survey reported an incidence of 265/100,000 children under the age of 5 in 2012, and suggested that the incidence of KD is still rising. Most patients are 6 months to 5 years old, although cases in older children and adults also occur. The male: female ratio is approximately 1.5.

Diagnosis

The diagnosis of KD is based on the presence of clinical features of persistent fever in combination with a polymorphous exanthema, cervical lymphadenopathy, non-purulent conjunctival injection, changes of the lips and oral cavity (including strawberry tongue, cracked lips, redness of the mucosae) and changes in extremities (swelling and redness of the palms, desquamation in the subacute phase). In the American Heart Association (AHA) guidelines, persistent fever is classified as ≥ 5 days, although fever for 4 or more days is now widely accepted in the presence of other symptoms. “Complete” KD is defined as fever and ≥ 4 out of the 5 symptoms. It is important to appreciate that criteria may present successively instead of simultaneously. The AHA has created an algorithm to increase the possibility of “incomplete” KD in case ≤ 3 criteria are present. This algorithm includes CA abnormalities on echocardiography and/or laboratory abnormalities. There is no diagnostic test for KD, and the diagnosis may be delayed or overlooked. To improve diagnosis, multiple new biomarkers have been studied, but none has so far proved specific for KD. Classification tools have been developed to aid in the differentiation between KD and other febrile illness, although the utility as a point-of-care diagnostic test remains unproven.
Etiopathogenesis

Although the etiology of KD is unknown, the current consensus is that it is likely caused by an infectious trigger initiating an abnormal immune response in genetically predisposed children. An infectious etiology is suspected due to the symptomatology of KD resembling common childhood infections, once-in-a-lifetime occurrence at young age (although second manifestations do occur), spatial and temporal clustering, and the clear seasonal pattern in high-incidence countries.

Infectious agents

Multiple viral infectious triggers have been suggested, including coxsackie virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus, chikungunya and cytomegalovirus. In fact, two recent studies showed that up to about half of all KD patients had one or more respiratory viruses detected by PCR, but their etiological role is unproven. Also, the possibility of a respiratory RNA virus has been suggested by ultrastructural studies of autopsy specimens. However, no virus has repeatedly been confirmed in KD studies.

Bacteria have also been suggested as the trigger of KD, with research mainly focusing on bacterial superantigens. Superantigens produced by bacteria are able to stimulate a large percentage of T cells by binding to the Vβ region of T-cell receptors on bacterial superantigens. Superantigens in KD remain unclear.

Immunological response

The encounter of a susceptible individual with the unknown agent probably leads to an (exaggerated) immune response involving innate and adaptive pathways. Multiple studies have been performed, both evaluating animal models and immune response in the peripheral blood as well as immune infiltration in the coronary arteries.

The general paradigm of the immune response is an imbalance between pro-inflammatory and anti-inflammatory pathways. For example, regulatory T cells, a subset of T cells limiting inflammation, have been shown to be important in the vascular inflammation. Also, the IL-1 signaling pathway is upregulated, with upregulated IL-1 pathway genes and increased IL-1 concentrations in peripheral blood of KD patients during the acute phase. Recently, it has become clear that inflammasomes, multiproteins that are part of the innate immune system, are induced by the NLRP3 gene and promote the production of IL-1β and IL-18, play a role in KD.

In the coronary arteries, immune infiltration of the arterial wall with neutrophils, CD8+ cytotoxic T cells, Ig-A producing plasma cells and macrophages have been found, accompanied by pro-inflammatory cytokines which may vary in proportion and contribution over time.

Genetics

Genetics are considered to contribute to susceptibility to KD, and probably to CAA and response to treatment. A number of genome-wide association studies (GWAS) have been performed. Apart from the GWAS, multiple studies have identified specific single-nucleotide polymorphisms (SNPs) in several genes. Most of these candidate genes have an immune regulatory function. Table 1 shows some of the key pathways and SNPs associated with KD susceptibility, CAA development and IVIG resistance.

In the first GWAS that resulted in a significant correlation with susceptibility to KD, we identified the major activating IgG receptor FcgRIIIA (CD32a) on immune cells and platelets, encoded by the FCGR2A gene at the FCGR2/3 gene cluster at chromosome 1q23. Following this study, Japanese and Taiwanese studies also confirmed this genetic association while at the same time characterizing CD40 and BLK, respectively as being associated with KD, as confirmed for BLK in Caucasian KD patients in a subsequent meta-analysis. CD40 is a protein expressed on antigen-presenting cells, such as dendritic cells, macrophages and B cells, and interacts with CD40L which is primarily expressed by activated T-cells and platelets. The function of FAM167A and BLK gene is yet to be investigated. The BLK gene encodes for tyrosine kinase, which is presumed to play a role in B cell signal transduction.

From alternative genetic studies (non-GWAS) other pathways were found to be involved, such as vascular endothelial growth factor (VEGF) and angiopoietin (ANGPT). ANGPT1 and angiopoietin receptor (TIE-2) promote cell survival and induces anti-inflammatory signals in contrast to ANGPT2 and TIE-2, which have a pro-inflammatory effect with VEGF acting as a co-factor. Also the transcription growth factor-beta (TGF-β) pathway may play an important role. TGF-β is important in T-cell activation and cardiovascular remodeling. One of the more recent and promising pathways involves the inositol-triphosphate 3-kinase (ITPKC) gene. ITPKC expression is part of a transmembrane signaling pathway with release of Ca2+ from intracellular storage. Initially, nuclear factor of activated T-cells (NFAT) was suggested to be involved in regulating the immune response in KD. The NFAT pathway is calcium-dependent, and when these cytosolic proteins become dephosphorylated, they translocate from the cytoplasm to the nucleus to initiate transcription of downstream target genes including...
### Candidate genes and pathways associated with disease susceptibility, CAA development and IVIG resistance

<table>
<thead>
<tr>
<th>Candidate pathway</th>
<th>Reference</th>
<th>Included cases/controls</th>
<th>Susceptibility to KD (gene)</th>
<th>Susceptibility to CAA (gene)</th>
<th>Susceptibility to IVIG resistance (gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABCC4</strong>&lt;br&gt;European descent (case-control), Australia, NL, USA, UK (family based)</td>
<td>Khor [44]</td>
<td>26 pedigrees, case-control=199/225, family based=1093 cases, 1621 parents, 198 siblings</td>
<td>Yes (ABCC4)</td>
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<td>x</td>
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<td><strong>ANGPT</strong>&lt;br&gt;Australia, US, UK (family based) and Dutch Caucasian (case-control)</td>
<td>Breunis [45]</td>
<td>462 complete trios, case-control: 123/171</td>
<td>Yes (ANGPT1)</td>
<td>Yes (ANGPT2)</td>
<td>x</td>
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<td><strong>Intergenic region BLK-FAM6A</strong>&lt;br&gt;Japanese</td>
<td>Onouchi [32] (GWAS) and Onouchi [32] (Replication 1), Onouchi [32] (Replication 2)</td>
<td>427/4399, 428/3378, 284/569</td>
<td>Yes (FAM67A-BLK)</td>
<td>x</td>
<td>x</td>
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<td>(Han) Chinese</td>
<td>Yan [46]</td>
<td>358/885</td>
<td>Yes (FAM67A-BLK)</td>
<td>No</td>
<td>x</td>
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<tr>
<td>Taiwanese</td>
<td>Lee [37] (GWAS) and Lee [37] (Replication), Lou [38] (GWAS)</td>
<td>622/1107, 261/550, 481/493</td>
<td>Yes (BLK)</td>
<td>x</td>
<td>x</td>
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<tr>
<td>Korean</td>
<td>Chang [38] (GWAS) and Chang [38] (Replication)</td>
<td>186/606, 188/498</td>
<td>Yes (BLK)</td>
<td>x</td>
<td>x</td>
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<tr>
<td>US (European, Asian, Hispanic, Mixed, African American, Native American, Samoan)</td>
<td>Onouchi [39]</td>
<td>593 trios</td>
<td>Yes (FAM67A-BLK)</td>
<td>x</td>
<td>x</td>
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<tr>
<td>European</td>
<td>Chang [40]</td>
<td>405/605</td>
<td>Yes (BLK)</td>
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<td><strong>CD40</strong>&lt;br&gt;Japanese</td>
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<td>428/3379, 470/378, 284/569</td>
<td>Yes (CD40L)</td>
<td>Yes (CD40)</td>
<td>x</td>
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<td>Han Chinese</td>
<td>Lou [43]</td>
<td>381/569</td>
<td>Yes (CD40)</td>
<td>Nominal association (CD40)</td>
<td>x</td>
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<tr>
<td>Taiwanese</td>
<td>Lee [44] (GWAS) and Lee [44] (Replication)</td>
<td>622/1107, 261/550</td>
<td>Yes (CD40)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>European</td>
<td>Shendre [45]</td>
<td>112 complete trios</td>
<td>Yes (CD40)</td>
<td>x</td>
<td>x</td>
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<tr>
<td><strong>FCGR2/3</strong>&lt;br&gt;Japanese</td>
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<td>Yes (FCGR2a)</td>
<td>Yes (FCGR3a)</td>
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<td>Duan [47], Khor [48]</td>
<td>428/493, 130/568</td>
<td>Yes (FCGR2a)</td>
<td>Yes (FCGR3a)</td>
<td>x</td>
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<tr>
<td>Taiwanese</td>
<td>Khor [49]</td>
<td>438/446</td>
<td>Yes (FCGR2a)</td>
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<td>x</td>
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<tr>
<td>Caucasian</td>
<td>Shrestha [50], Shrestha [51]</td>
<td>170/359, 156 trios, 75 single parent-child</td>
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<td>Yes (FCGR3a)</td>
<td>x</td>
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<tr>
<td>European</td>
<td>Khor [52] (GWAS)</td>
<td>405/605</td>
<td>Yes (FCGR2a)</td>
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## Table 1 Continued

<table>
<thead>
<tr>
<th>Candidate pathway</th>
<th>Reference</th>
<th>Included cases/controls</th>
<th>Susceptibility to KD (gene)</th>
<th>Susceptibility to CAA (gene)</th>
<th>Susceptibility to IVIG resistance (gene)</th>
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<td><strong>ITPKC</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Japanese</td>
<td>Onouchi</td>
<td>223/318</td>
<td>Yes (ITPKC)</td>
<td>Yes (ITPKC)</td>
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<td>Onouchi</td>
<td>130/588</td>
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<td></td>
<td>Onouchi</td>
<td>257/572</td>
<td>x</td>
<td>x</td>
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<tr>
<td></td>
<td>Onouchi</td>
<td>546/947</td>
<td>Yes (ITPKC)</td>
<td>Yes (ITPKC)</td>
<td>Yes (ITPKC)</td>
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<tr>
<td></td>
<td>Chi</td>
<td>385 (of which 158 210)</td>
<td>No</td>
<td>No</td>
<td>x</td>
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<tr>
<td></td>
<td>Lin</td>
<td>280/492</td>
<td>Yes (ITPKC)</td>
<td>No</td>
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<td></td>
<td>Kuo</td>
<td>341/1190</td>
<td>Yes (ITPKC)</td>
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<td>Khor</td>
<td>999/2781</td>
<td>Yes (ITPKC)</td>
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<tr>
<td></td>
<td>Khor</td>
<td>385/569</td>
<td>No*</td>
<td>Yes (ITPKC)</td>
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<td></td>
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<td></td>
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<tr>
<td><strong>European</strong></td>
<td>Khor</td>
<td>425/5252 (105)</td>
<td>Yes (ITPKC)</td>
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<td>x</td>
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<td></td>
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<tr>
<td><strong>US</strong></td>
<td>Onouchi</td>
<td>209/205</td>
<td>Yes (ITPKC)</td>
<td>Yes (ITPKC)</td>
<td>Yes (ITPKC)</td>
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<td><strong>TGFB pathway</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Japanese</td>
<td>Cho</td>
<td>105/303</td>
<td>Yes (SMAD5)</td>
<td>No</td>
<td>x</td>
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<tr>
<td>Han Chinese</td>
<td>Peng</td>
<td>392/422</td>
<td>Yes (TGFB2, SMAD3, ADAM17)</td>
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<tr>
<td>Taiwanese</td>
<td>Kuo</td>
<td>385/569</td>
<td>Yes (SMAD3)</td>
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<td>No</td>
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<td>Korean</td>
<td>Choi</td>
<td>105/500</td>
<td>Yes (TGFB2)</td>
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<td>European descent</td>
<td>Shimizu</td>
<td>128/159</td>
<td>Yes (TGFB2)</td>
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<td>Japanese</td>
<td>Kariyazono</td>
<td>103/144</td>
<td>x</td>
<td>Yes (VEGF)</td>
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<tr>
<td>Taiwanese</td>
<td>Hsueh</td>
<td>97/96</td>
<td>Yes (VEGF)</td>
<td>x</td>
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<tr>
<td>Australia, US, UK</td>
<td>Breunis</td>
<td>462 complete trios,</td>
<td>Yes (VEGFA)</td>
<td>Yes (VEGF2)</td>
<td>x</td>
</tr>
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<td>(family-based),</td>
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<td>case control: 123/171</td>
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<tr>
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<td>Breunis</td>
<td>170/300</td>
<td>Yes (VEGF)</td>
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a. Numbers after quality control, starting numbers: 627/1118.
b. Numbers after quality control, starting numbers: 222/600.
c. Significant difference between male patients with CAA (n=51) compared with male patients without CAA (n=185) and with controls, not in female patients.
d. rs48149465 has protective effect for CAA formation in KD patients.
e. Numbers after quality control, starting numbers: 477/137. f. Only significant taken in all ethnic groups (White and Asian combined).
f. Numbers after quality control, starting numbers: 578/7146, cases/controls.
g. ITPPKN SNP not genotyped in 584 controls of first cohort.
h. A 2-locus model in combination with a SNP in CASP3 was also a significant association with both CAA development and IVIG resistance.
i. CAA in >5 mm or in children over 5 years of age, diameter of at least 1.5 times adjacent segment.
j. Two SNPs found but significance disappeared after correction for multiple testing.
k. Total included trios in study = 740 which is 155 of non-European descent, combined analyses.
l. Only 1 SNP remained significant after Bonferroni correction.
m. Different SNPs found in different controls of any of the SNP colocalized to the same region of each of the 3 genes (TGFB2, TGFBR2, and SMAD3).

**Notes:**

for cytokines such as IL-2, IL-10, and IFNγ. Stimulation of T-lymphocytes accommodates the release of inositol-triphosphate (IP3), which increases intracellular Ca²⁺ through the endoplasmic reticulum (ER) and mitochondria creating a site of contact between the ER and mitochondria called the Mitochondria-Associated ER Membrane (MAM). ITPKC phosphorylates IP3 to IP4 and modulates the abundance of IP3 and influences the calcium signaling. Inositol triphosphate receptor (IP3R) forms a bridge between the Endoplasmatic Reticulum (ER) and mitochondria creating a site of contact between the ER and mitochondria called the Mitochondria-Associated ER Membrane (MAM). NLRP3 is an inflammasome that forms at or close to the MAM upon cellular activation and ER stress and plays a pivotal role in the cleavage of pro-IL-1β into IL-1β and its subsequent secretion. The ER releases calcium into the cytosol and into mitochondria through (a.o.) the IP3R, which is a calcium channel, to which IP3 as an agonist binds to induce calcium release. IP3R binds via glucose-regulated protein 75 (GRP75) with the mitochondrial voltage dependent anion channel 1 (VDAC1) which may cause mitochondrial stress and leakage of reactive oxygen species (ROS), both important for inflammasome activation. Macrophages activate via their Toll-like receptors (TLRs) or G-protein coupled receptors several signaling pathways, that result in IP3 formation, NF-κB activation and/or ER stress.

**FIGURE 1** The role of IP3 and ITPKC in calcium signaling

- **a.** ITPKC phosphorylates IP3 to IP4 and modulates the abundance of IP3 and influences the calcium signaling.
- **b.** Nuclear factor of activated T-cells (NFAT) are regulated by calcium signaling and enter the nucleus when dephosphorylated, there it activates cytokine transcription namely IL-2, IFNγ in T-cells and Pro-IL-1β, Pro-IL-18 in macrophages.

The current treatment for KD is a high-dose of 2 mg/kg intravenous immunoglobulins (IVIG), given over 8-12 hours⁶. The main goal of treatment is prevention of the development of CAA. Hypothesis on the mechanisms of efficacy of IVIG include immune modulation of T-regulatory cells, neutralization of the etiologic agent and reduction of cytokine production⁷. Treatment with IVIG significantly reduces the incidence of CAA⁸. IVIG is preferably given within the first 10 days after disease onset⁹. Apart from IVIG, high-dose aspirin is advised by the AHA, although evidence for further risk-reduction for CAA is lacking⁹,¹⁰.

**Treatment**

The majority of patients respond rapidly to IVIG, yet approximately 10-20% of all patients do not respond or have recurrent fever within 36-48 hours after IVIG. These children have a higher risk of developing CAA¹¹. In Japan, risk-scores have been developed to identify patients with a higher risk of IVIG resistance¹²-¹⁴. Unfortunately, these risk-scores do not perform adequately in Western, ethnically mixed and in Chinese populations¹⁵-¹⁷. A possible method to decrease IVIG resistance is to intensify the initial treatment. A recent meta-analysis showed a beneficial effect of adding corticosteroids to the initial treatment with IVIG, yet this effect was only found in Japanese studies and not in two studies conducted in the US¹⁸. Burns et al showed that adding infliximab to initial IVIG-treatment did not decrease treatment-resistance but did decrease the number of days with fever and inflammation parameters 24 hours after treatment¹⁹.

**Adjunctive use of treatment**

Children who do not respond to IVIG require additional anti-inflammatory treatment. A second dose of IVIG is commonly advised, particularly in patients who have partially responded. Furthermore, corticosteroids are still commonly advised. The above leading to disease severity⁴⁰. Alphonse et al suggested that the role of ITPKC is not T-cell-mediated but more monocyte/macrophage-dependent in its impact⁴¹. They showed that ITPKC influences NLRP3 activation through intracellular calcium levels leading to an increased IL-1β and IL-18 production. Khor et al performed a global meta-analysis of SNP rs2493229 in ITPKC of all performed studies, including GWAS data, showing strong evidence for association with KD (p=8.28 × 10⁻¹²⁴)

Other pathways and candidate genes have, sometimes inconsistently, been implicated including ATP-binding cassette, subfamily C, member 4 (ABCC4), interleukines-4, -10 and -18, chemokine receptors, tumor necrosis factor-α and, even more variably, different regions of the human leukocyte antigen (HLA) region.
mentioned meta-analyses showed no significant benefit for either of these approaches when used as rescue treatment. In 2012, we published the first case-report successfully using the IL-1 receptor antagonist anakinra for refractory KD. Since then, additional clinical trials have been instigated to investigate both efficacy and safety of this IL-1 inhibitor. Other secondary treatment possibilities are infliximab (TNF-α inhibitor), cyclosporine (calcineurin inhibitor), and statins, yet efficacy remains to be investigated.

Additional treatment
After normalization of temperature, the AHA advises ongoing aspirin in a low dose until no evidence of CA dilation are present at about 6 weeks after the acute illness. If CAA are present and persisting around that time, aspirin is continued as anti-thrombotic therapy. In case of large (around a z-score ≥ 10) or complex abnormalities additional anticoagulation therapy should be administered to prevent clotting due to turbulence in these pro-coagulatory large coronary artery lesions.

Coronary artery aneurysms
Multiple criteria have been used for diagnosis of CAA. The criteria of the Japanese Circulation Society (JSC) state that an aneurysm is an artery of >3 mm in a child under the age of 5 and an artery of >4 mm in a child ≥5 years or when an arterial segment is 1.5 times its adjacent segment. A giant CAA is classified as ≥8 mm or >4 times its adjacent segment. Conversely, over the past years it has become clear that z-scores, diameters adjusted for basal-surface-area may be better indication of abnormality. Multiple z-score classifications exist. Unfortunately, the z-scores using different classifications can vary, mainly at larger dimensions. The threshold for abnormality is a z-score ≥ 2.5. A small-sized CAA has a z-score of 2.5–5, a medium-sized CAA of 5–10 and a giant CAA of ≥ 10.

Risk factors for CAA have been inconsistently reported but include a male gender, a young age (<1 year), an incomplete disease presentation, IVIG resistance and the duration of fever.

Imaging of CAA
Multiple imaging techniques exist for the follow-up of patients after KD and in particular children with CAA. Echocardiography is a non-invasive method to image the coronary arteries, used in the acute phase of KD as well as during follow-up. With this non-invasive method, it is possible to evaluate the anatomy of the coronary arteries, myocardial function and valve abnormalities. Nevertheless, it is impossible to visualize the distal coronary arteries with echocardiography. The gold standard for coronary anatomy is a conventional angiography, though this technique is invasive and exposes the patient to radiation. The role of cardiac MRI (cMRI) has been established over the past years. Using this modality, evaluation of the anatomy as well as cardiac function is possible. The disadvantage of cMRI is the need for anesthesia in younger children. CT-angiography is an alternative. Although conventional CT-angiography carries a high burden of radiation exposure, the new low-radiation dose CT scanning machines are becoming more widely available, which decreases the radiation burden significantly showing good resolution capacity in a small study.

In Figure 2, a MRI and CT image of a normal and a giant CAA are depicted.

The AHA and JCS have both published guidelines on the follow-up of patients after KD. Recently we proposed a pathway to follow-up patients after KD based on the worst-ever z-score. This pathway includes a cMRI (with or without adenosine stress testing according to the CAA status) during adolescence for all patients to visualize the complete coronary tree and a more intensive follow-up for patients with CAA (Figure 3).

Natural history of CAA
Many CAA regress to a normal-sized lumen, mainly within the first 5 years. The likelihood of regression seems to be highly dependent on the original CAA size. While the lumen diameter may return to normal, it has become apparent that the vascular wall is...
CHAPTER 2
DISSECTING KAWASAKI DISEASE – A STATE-OF-THE-ART REVIEW

INTRODUCTION


a. When information is lacking about coronary arterial aneurysms (CAA) status, calcium score may be indicated as a screening method. If positive, a CMRI with adenosine should be performed.

b. Long-term follow-up (cardiovascular counseling) of risk group 1 may be dictated by national health care policies and future studies.

c. According to the availability and experience of a center with (low-dose) CT angiography.

d. Which of the different revascularization options improves prognosis best is unclear to date.

e. Additional tests to evaluate for progression to stenotic lesions.

Consequences of CAA

(Giant) CAA may have serious long-term consequences. Apart from thrombosis within the CAA and perfusion abnormalities after the CAA, there is an increased risk of stenosis just proximal or distal to the CAA. In a large study of 245 Japanese patients with giant CAA (>8 mm), Tsuda et al found 10-, 20-, and 30-year event-free survival rates of 64%, 48% and 36%, respectively. Fifteen patients died during follow-up. In another study of 76 Asian patients with giant CAA (>8 mm), 10-, 20-, and 30-year survival rates of 95%, 88% and 88% were reported. In a recent study by Friedman et al., 21 major adverse cardiac events (MACE) took place in 90 patients with giant CAA at diagnosis (z-score ≥ 10). This indicates that patients with giant CAA are at considerable risk for MACE and risk continues to exist years after the acute phase of KD.

The risk for patients with small-medium-sized CAA is less clear. In a study by Chih et al., only 1/51 patients with medium-sized CAA (>4-8 mm) experienced ischemia during a median follow-up time of 47 months, although an additional 8 patients had stenosis and 4 patients developed calcification. No patients with small-sized (localized dilatation with ≤ 4 mm diameter) CAA experienced MI. In the study by Friedman et al, no patients with small (z-score 2.5-5) or medium-sized CAA (z-score 5-10) experienced any MACE. However, longer term studies will be needed to establish the long-term event rate in patients with small or medium sized CAA.

In patients with persisting CAA, calcifications are likely to develop as shown by a recent study looking at CT calcium scoring. The data suggested this only occurs from approximately 10 years onwards following KD.

If thrombosis, stenosis or abnormal blood flow in the CAA leads to a cardiac event or signs of ischemia, KD patients may need cardiac intervention. Thrombolytic treatment has been reported to be effective in treating fresh thrombus within giant CAA and in emergency management of ischemia due to thrombus. Percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) can be used to revascularize the artery when stenotic lesions are present. CABG is used more often in multi-vessel disease. Percutaneous transluminal angioplasty may be complicated by the need of high balloon pressures in patients who have already developed occlusions.
calcification. Two studies evaluating the difference between these two methods found that reinterventions were significantly higher in the PCI group. Available data are scarce on risk during pregnancy for women after KD. In a case series including 10 women who delivered 21 babies, none of the women experienced cardiovascular complications during pregnancy, including 4 women with CAA, MI and CABG in the past. A following comprehensive review, 56 women with 81 deliveries were described and cardiovascular complications were reported in 7 cases, including two MIs during pregnancy.

Longer term studies will be needed to define the longer term risk of CAA. However the significant proportion of patients developing thrombosis within giant CAA, or suffering ischemic events due to stenosis suggests that all patients with significant aneurysms following KD need life time follow up and are at risk of cardiac complications long term.

General cardiovascular risk
Apart from the increased cardiovascular risk due to persisting or regressed CAA, it is uncertain whether the vasculitis itself causes an increased cardiovascular or atherosclerotic risk at a later age. As most patients have not been followed long enough to evaluate the long-term natural course of the disease, multiple studies have focused on the use of surrogate markers for cardiovascular disease such as flow-mediated dilation, stiffness index and carotid intima-media thickness (cIMT). Two reviews showed that most of these studies are small, lacking quality and there was significantly heterogeneity between studies. Nevertheless, results suggested that surrogate markers were increased in CAA-positive but not in CAA-negative patients. In a follow-up study evaluating cIMT, we found that patients with giant CAA have a trend towards an increased cIMT at a later age, whereas in children without any coronary artery enlargement, their cIMT was initially increased but normalized to control values over time. The results suggest that long-term effects of KD are not caused by atherosclerosis, as one would expect the differences in cIMT to increase compared with control measurements. This is in concordance with a postmortem study in which multiple growth factors were seen in the smooth muscle cells and intima layer of the coronary arteries, but no fatty streaks as seen atherosclerosis, distinguishing “KD vasculopathy” from atherosclerosis.

Quality of life and behavior
Multiple studies have investigated the cognitive and behavioral outcome after KD. Baker et al. studied 110 KD children, and found similar psychosocial and physical summary scores as a US population sample using a parent-completed questionnaire. Only patients with giant CAA had a lower mean physical score. Parents did however report lower health perception. King et al. studied 38 KD patients and found no effect on cognitive or academic performance, but parents rated their children as having more internalizing and attentional behavior problems than controls. Carlton-Conway et al. found that 40% of their patients showed internalizing scores in the clinical range as reported by parents, which was significantly more than their hospital controls who stayed in the hospital for a short period and had undergone cardiac catheterization. Nishad et al. found no difference in social adaption, cognitive function and behavioral function in 20 children. Muta et al. studied 350 adolescents and young adults, including 19 patients with giant CAA and found significantly higher health-related quality of life (HRQOL) scores compared to national norms. Two studies from our center showed significantly lower scores on several HRQOL scales in children under 5, when reported by their parents. However, self-report by the older KD children did not show any significant difference with controls. Moreover, parental perceptions of child vulnerability were significantly increased when compared to reference groups of Dutch parents.

Conclusion
Although many aspects of KD are still unknown, there is increasing knowledge on the origin and treatment of KD as well as the development and classification of CAA. Since children with previous KD are entering adulthood, long-term follow-up, with appropriate imaging modalities and awareness of the long-term effects, is increasingly important.
References


PART I

CARDIOVASCULAR RISK AND THE FOLLOW-UP OF PATIENTS AFTER KAWASAKI DISEASE
Peripheral endothelial (dys) function, arterial stiffness and carotid intima-media thickness in patients after Kawasaki disease: a systematic review and meta-analyses

S.M. Dietz, C.E.A. Tacke, B.A. Hutten, T.W. Kuijpers

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PART I

Introduction
Kawasaki disease (KD) is a pediatric vasculitis mainly affecting children under the age of 5. Coronary artery aneurysms (CAA) develop in 25% of untreated and 5-15% of patients treated with intravenous immunoglobulins, making it the most common cause of pediatric acquired heart disease in the Western world.

It can be hypothesized that, due to the previous systemic vasculitis, patients with KD have an increased risk for cardiovascular disease (CVD) at a later age, apart from the presence or absence of CAA. This hypothesis is difficult to test since KD was first described less than 50 years ago and therefore most of the KD patients are too young to have experienced cardiovascular events.

In recent years, several non-invasive surrogate markers of CVD risk have become available.

Endothelial dysfunction can be measured by flow-mediated dilatation (FMD), nitroglycerin-mediated dilation (NMD) or peripheral arterial tonometry (PAT). Peripheral arterial stiffness can also be an indicator of increased CVD risk. It can be measured by pulse wave velocity (PWV) or by the beta stiffness index (SI). Furthermore, structural changes in the arterial wall can be found by measuring the carotid intima-media thickness (cIMT), well-established surrogate marker of atherosclerosis and subsequent predictor of cardiovascular events.

The aim of this study was to systematically review and meta-analyse the existing literature regarding surrogate markers for CVD risk in KD patients.

Methods
Search strategies
We conducted a systematic literature search of Medline (1966-September 2014) and Embase (1980-September 2014) for studies addressing KD and surrogate markers of cardiovascular risk (i.e. endothelial dysfunction, peripheral arterial stiffness and cIMT). We used two domains of MeSH terms and free text words combined by ‘AND’, and in each domain the terms were combined by ‘OR’. The first domain contained terms of KD (including all synonyms and abbreviations), and the second contained terms of surrogate markers of cardiovascular risk (including all synonyms, abbreviations and free word text such as ‘carotid intima-media thickness’, ‘vascular stiffness’, ‘endothelial dysfunction’, ‘flow-mediated dilatation’, ‘pulse wave velocity’, ‘peripheral arterial tonometry’). The complete protocol is registered in the Prospero database under CRD42014005706, the PRISMA checklist and Medline electronic search strategy are added as supplemental files (St checklist and St file).
Study selection and quality assessment

We selected those original studies that reported on surrogate markers of cardiovascular risk (i.e. endothelial dysfunction [FMD, NMD, PAT], vascular stiffness [PWV, SI] and cIMT) in KD patients. Studies were excluded if healthy control groups were not available within the same studies, if lipid-lowering medication was used when measuring subjects, or if data contained preliminary results. Furthermore, because of the possible influence of the acute inflammation, studies measuring patients within 6 months after the acute phase were excluded. Language restrictions were not imposed. The selection process was divided into three successive stages: title-, abstract- and manuscript selection. Two investigators (SD and CT) independently determined eligibility of the retrieved studies, according to predefined criteria. Using an adjusted version of the Newcastle-Ottawa scale for observational studies (StTable: Quality assessment criteria), the same investigators assessed the methodological quality of the eligible studies. Selection of patients and controls, comparability, and outcome measurements were evaluated. Disagreements were solved by discussion, and if necessary, by the opinion of a third reviewer.

Data extraction

Using a predetermined form, two investigators (SD and CT) independently extracted data of the eligible articles. Information was collected on study characteristics (study design, country and sample size). The CAA-classification used was retrieved and the number of CAA-positive patients was noted based on whether patients ever had CAA (worst-ever CAA-score). In addition, the following characteristics of participants were extracted: gender, age, blood pressure, BMI, and treatment during the acute KD phase. Outcome measurements were, if possible, collected for the control- and the whole KD group, as well for the CAA-negative and CAA-positive group. When data were missing, the corresponding authors were emailed to request the information.

Statistical analysis

When studies described multiple CAA-positive groups based on severity, we calculated pooled estimates of the mean and standard deviation (SD) values for the overall CAA positive group. The same was done when cIMT was described separately for the left and the right carotid artery. When p-values were not provided, but mean, SD and numbers were reported, we calculated whether there was a significant difference between patients and controls using review manager software, version 5.2 (Cochrane Collaboration). We used the same software to create forest plots for cIMT, FMD and SI in KD patient compared to controls and in CAA positive patients compared to controls, with study-level effect sizes calculated as absolute mean differences. We measured the proportion of between-study differences not attributable to chance with the I² statistic. We considered values of 25-50%, 50-75% and ≥75% to indicate low, moderate and high heterogeneity, respectively. Only when heterogeneity was low or moderate (≤75%), pooled estimates of the summary mean difference were computed using the random-effects model according to the method of DerSimonian and Laird7. A Z-test was performed to test the overall effect.

When studies with overlapping inclusions were present, the largest study was used for the forest plot and if applicable, the pooled estimate.

Heterogeneity was explored by a sensitivity analyses for all analyses. Furthermore, for the whole KD group, a meta-regression analysis using the random effects, methods of moments approach was performed, with study characteristics as covariates. We looked at ‘time since KD’, ‘percentage of IVIG-treated patients’ and ‘percentage of CAA-positive patients’. We did not perform meta-regression analyses on the CAA-positive groups because covariables were usually not described separately for CAA-positive groups in most studies. We used comprehensive meta-analyses software (version 2) to execute the meta-regression analyses.

We did not perform meta-analyses on the data of CAA-negative patients because not all studies used the same CAA-criteria. This implies that a child might have no enlargement according to one classification but does have an aneurysm according to the other. All CAA-positive patients have enlargement according to at least one classification system.

Results

Description of studies

Our search retrieved 621 articles. After scanning titles and/or abstracts, we excluded 586 studies. Of the remaining 35 articles, five were excluded based on the whole article. Hence, 30 studies remained for final inclusion (Figure 1). Table 1 shows the characteristics of these studies.

Four studies were cohort studies with a maximal follow-up of 6 months8-11 and the remainder had a cross-sectional study design. The total number of subjects per study varied from 22 to 253. The mean age of KD patients ranged from 6.5 to 31.5 years. Although the criteria used were not clearly stated in all manuscripts, most studies defined CAA according to Japanese criteria or by z-scores12,13. Two articles used deviating classifications14,15. Most studies reported the worst-ever CAA-score, two studies reported the CAA-status 30 days after beginning of the disease16,17 and in three studies it was not clear which status was reported17,19. Some research groups invited the same patients for different studies, which created overlap of inclusions between studies20,21,22. Table 2 shows a summary of findings.
TABLE 1 Characteristics of studies

<table>
<thead>
<tr>
<th>First author, year</th>
<th>Country</th>
<th>Kawasaki patients</th>
<th>Controls</th>
<th>Reported outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dhillon, 1996</td>
<td>UK</td>
<td>15% IVIG 20% CAA+</td>
<td>12 (60) 11.3</td>
<td>FMD, NMD</td>
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<td>Silva, 2001</td>
<td>Canada</td>
<td>33% CAA+ 14% CAA-</td>
<td>17 (30) 14.3</td>
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</tr>
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<td>Noto, 2001</td>
<td>Japan</td>
<td>58% CAA+ 12% CAA-</td>
<td>12 (60) 9.8</td>
<td>SI</td>
</tr>
<tr>
<td>Deng, 2002</td>
<td>China</td>
<td>73% CAA+ 24% CAA-</td>
<td>28 (75) 3.4</td>
<td>SI</td>
</tr>
<tr>
<td>Cheung, 2004</td>
<td>China</td>
<td>97% CAA+ 5% CAA-</td>
<td>35 (80) 7.8</td>
<td>PWV</td>
</tr>
<tr>
<td>Cheung, 2004</td>
<td>China</td>
<td>92% CAA+ 9% CAA-</td>
<td>35 (70) 6.9</td>
<td>PWV</td>
</tr>
<tr>
<td>Ikemoto, 2005</td>
<td>Japan</td>
<td>100% CAA+ 50% CAA-</td>
<td>20 (50) 10.6</td>
<td>PWV</td>
</tr>
<tr>
<td>Kadono, 2005</td>
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<td>NA CAA+ 24% CAA-</td>
<td>11 (25) 5.8</td>
<td>PWV</td>
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<td>Chili</td>
<td>100% CAA+ 10% CAA-</td>
<td>11 (25) 10.6</td>
<td>FMD</td>
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<td>McCrindle, 2007</td>
<td>Canada</td>
<td>64% CAA+ 52% CAA-</td>
<td>30 (50) 14.9</td>
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<td>Cheung, 2007</td>
<td>China</td>
<td>90% CAA+ 5% CAA-</td>
<td>22 (55) 7.4</td>
<td>SI</td>
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<td>Huang, 2008</td>
<td>Taiwan</td>
<td>100% CAA+ 10% CAA-</td>
<td>11 (25) 12.9</td>
<td>FMD</td>
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<td>First author, year</td>
<td>Country</td>
<td>Kawasaki patients</td>
<td>Controls</td>
<td>Reported outcome</td>
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<tr>
<td></td>
<td></td>
<td>IVIG (%)</td>
<td>No/CAA+ (%</td>
<td>Age (yrs.)</td>
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<tr>
<td>Niiboshi, 2008 13</td>
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<td>6</td>
<td>35 (15/20)</td>
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<td>Cheung, 2009 12</td>
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<td>75</td>
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<td>13±2.6</td>
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<td>Liu, 2009 14</td>
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<td>41 (21/20)</td>
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<td>Gupta-Malhotra, 2009 91</td>
<td>USA</td>
<td>36</td>
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<td>Lee, 2009 14</td>
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<td>25 (25/0)</td>
<td>12.6±2.0</td>
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<tr>
<td>Noto, 2009 16</td>
<td>Japan</td>
<td>52</td>
<td>35 (35/0)</td>
<td>20.5±2.9</td>
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<tr>
<td>Duan, 2011 10</td>
<td>China</td>
<td>97</td>
<td>31 (31/0)</td>
<td>6.2±3.4</td>
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<tr>
<td>Noto, 2012 15</td>
<td>Japan</td>
<td>64</td>
<td>18 (18/0)</td>
<td>17.2±5.3</td>
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<td>Pinto, 2013 13</td>
<td>Portugal</td>
<td>100</td>
<td>19 (0/19)</td>
<td>21±6</td>
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<td>100</td>
<td>24 (9/25)</td>
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<td>Selamet Tierney, 2013 12</td>
<td>USA</td>
<td>93</td>
<td>203 (47/156)</td>
<td>16.7±2.4</td>
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<table>
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<tr>
<th>First author, year</th>
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<th>Kawasaki patients</th>
<th>Controls</th>
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<tr>
<td>Duan, 2014 10</td>
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<td>100</td>
<td>13 (13/0)</td>
<td>5.8±2.1</td>
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<td>Cho, 2014 18</td>
<td>Korea</td>
<td>91</td>
<td>68 (19/49)</td>
<td>CAA+; 8.00±2.84</td>
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<td>Laurito, 2014 19</td>
<td>Italy</td>
<td>0</td>
<td>14 (7/7)</td>
<td>10.0±2.3</td>
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<tr>
<td>Oguri, 2014 10</td>
<td>Japan</td>
<td>81</td>
<td>75 (11/64)</td>
<td>8.2±2.8</td>
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<tr>
<td>Singh-Meena, 2014 10</td>
<td>India</td>
<td>100</td>
<td>27 (10/17)</td>
<td>8.2±2.6</td>
</tr>
</tbody>
</table>

Values represent mean ± SD unless otherwise indicated. * Median (range). † Mean (range). ‡ IVIG for 5 days § Median (IQR). ¶ p<0.05 for age or sex when KD group is compared to control group. FMD, Flow-mediated dilation; CAA, Coronary arterial aneurysm; IVIG, Intravenous immunoglobulin; CAA, Coronary artery aneurysm; FMD, Flow-mediated dilation; NA, not available; NMD, Nitroglycerin-mediated dilation; cIMT, Carotid intima-media thickness; SI, stiffness index; PWV, Pulse wave velocity.
Quality of studies
The results of the quality assessments are shown in Figure 2. All studies were found to have methodological limitations; the overall scores ranged from 4 to 11 (maximum of 16, S2 table: Quality assessment per study). Most limitation arose from representativeness of cases, definition of controls and lack of adjustment for potential confounding factors.

Endothelial dysfunction
Flow-mediated dilation
A total of 15 studies reported on FMD (Table 2). Eleven studies showed a significantly decreased FMD in patients after KD as compared to controls, with a mean or median difference ranging from -9.7% to -2.7%. Four studies showed no statistical significant difference.

In meta-analyses, after excluding two studies reporting median instead of mean FMD, an extensive heterogeneity between the remaining 13 studies was found (I²=89%). Therefore, we did not pool the results (Figure 3A). Subsequently, heterogeneity was explored by sensitivity analyses and by means of meta-regression analysis. None of the three predefined covariables were shown to be of significant influence on heterogeneity.

Flow-mediated dilation and CAA status
Seven studies compared CAA-negative patients to controls; four studies found a significantly decreased FMD, whereas three studies did not find any difference (Table 2).

CAA-positive patients were found to have a significantly decreased FMD compared to controls in 10 out of 11 studies. Two studies reported median FMD. When analyzing the nine studies describing mean FMD, a high heterogeneity was found (I²=89%, Figure 3B). If the small study by Laurito et al.39, which included only seven patients with mild transient CAA, was excluded from the analyses, moderate heterogeneity was calculated (I²=59%). Combining the remaining eight studies, a statistically significant decreased FMD was observed in patients after KD (mean difference (-6.06%, 95%CI: -7.76% to -5.47%).

Nitro-glycerine-mediated dilation
In addition to FMD, seven studies measured NMD (Table 2). None of the studies found a significant difference between patients after KD and controls, neither when looking at the groups as a whole nor when looking at CAA-negative or CAA-positive patients.

Peripheral arterial tonometry
All three studies reporting PAT used the Endo-PAT device to measure the reactive hyperemia index (RHI) or Endo-PAT Index. Selamet Tierney et al., studying 203 patients,
## TABLE 2 Summary of findings

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<th>Mean CAA- and CAA+ pt.</th>
<th>P *</th>
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* p whole KD group vs controls, † p CAA-negative patients vs controls, ‡ p CAA-positive patients vs controls, § p<0.001 mildly rate and severe aneurysms vs controls. || Median (IQR), # based on ANOVA, p>0.05 for male patients, p not significant for female patients, †† in original article stated as m/s, †† z-score > 3.
NS, non-significant (as reported in original articles). BA, Brachial artery; cIMT, carotid intima-media thickness; FMD, flow-mediated dilatation; LCCA, Left common carotid artery; n-d, non-dominant; NMD, nitroglycerin-mediated dilatation; PAT, peripheral arterial tonometry; PWV, pulse wave velocity; RCCA, Right common carotid artery; SI, stiffness index.
Italic numbers are calculated using the data in the original articles.
found no difference in Endo-PAT index between patients and controls. A similar result was reported by Tobayama et al. Pinto et al. found a significantly lower RHI in their CAA-negative patients (Table 2).

Vascular stiffness

Stiffness index

A total of 10 studies studied the SI of the carotid artery (Table 2). Seven studies found a significant increased SI in patients compared to controls, while three studies found no difference. After excluding two studies because of overlapping inclusions in the meta-analysis and one study because they reported the median, there was high heterogeneity between the remaining studies (I²=81%, Figure 4A).

A meta-regression analysis showed an independent positive association between the percentage of CAA-positive patients and the mean difference in SI (p=0.001), which may partly explain the high heterogeneity.

Stiffness index and CAA status

Five out of six studies reporting on CAA-negative patients did not find a significant difference in SI of patients compared to controls.

Nine studies studied CAA-positive patients. Seven studies found a significant difference in SI between CAA-positive patients and controls, while two studies did not. Two studies were not included in the meta-analysis because of overlapping inclusions and one because it measured the median instead of mean. High heterogeneity was found between the remaining six studies (I²=88%; Figure 4B). After excluding the small study of Gupta-Malhotra with only nine patients with early transient CAA, heterogeneity decreased to 60%. When pooling the data of the remaining studies, a history of CAA was associated with a significantly increased SI of 0.67 (95% CI 0.38-0.96).

Pulse wave velocity

PWV in patients after KD was reported in six studies. Cheung et al. performed three of these, all showing an increased brachial-radial PWV in CAA-positive and CAA-negative patients compared to controls. These results were similar to the results of Cho et al.

Two studies measured brachial-ankle PWV (baPWV). Lee et al. found an increased PWV in their CAA-positive patients compared to controls. Of note, their patient group was significantly younger than the control group, although one would expect the difference to be greater as PWV increases with age. Niboshi et al. found a significantly faster PWV in adult male but not in female KD patients as compared to controls.

Carotid intima-media thickness

A total of 15 studies reported on cIMT in patients after KD (Table 2). The studies reported the cIMT of the right common carotid artery (CCA), the left CCA or the mean of both. Mean cIMT was reported in eight, while maximum cIMT was reported in seven studies. Seven studies reported a significantly increased cIMT, seven studies showed no significant difference and one study showed a decreased cIMT as compared to controls.

In the meta-analysis, studies measuring mean cIMT and maximum cIMT were analyzed separately (Figure 5A and 5B). When analyzing the studies measuring mean cIMT, one study was excluded because of overlapping inclusions. We found moderate heterogeneity in the remaining seven studies (I²=51%). When pooling the data of these studies, a mean difference of 0.01 mm (95% CI 0.00 to 0.02 mm) was found between patients and controls (Figure 5A). It was remarkable that in the two studies showing a thinner cIMT in KD patients, the control group was or seemed to be significantly older. In meta-regression analyses none of the three predefined covariables was of significant influence on the heterogeneity.

Two studies measuring maximum cIMT were not included in the forest plot because of overlapping inclusions (Figure 5A) and one because it measured the median instead of mean. High heterogeneity was found in adult male but not in female KD patients as compared to controls.

Carotid intima-media thickness and CAA-status

Seven studies described CAA-negative patients and only one found an increased cIMT in patients compared to controls (Table 2).
PART I

Selamet Tierney et al. found a significantly thicker left cIMT in patients with a history of giant aneurysms, whereas patients with a history of ectasia, small or medium CAA did not show this phenomenon.

Discussion

Our systematic review summarizes 30 studies on surrogate markers for CVD risk in patients after KD compared to unaffected controls. FMD and SI were increased in most studies, being more pronounced in CAA-positive patients. Mean cIMT in the whole KD-group and the CAA-positive group did not seem to be increased while data on maximum cIMT were inconclusive. The results of this review have to be interpreted with care due to methodological limitations and substantial heterogeneity between studies.

Quality of studies

Most studies had important methodological limitations. First, CVD risk is dependent on many factors. It has been shown that cIMT and FMD are dependent on life-style factors such as social-economic status and physical activity. Therefore, a suitable control group is vital, which many studies failed to include or describe. Secondly, factors such as age, gender, blood pressure and BMI are known to influence surrogate markers. These variables should be identified and adjusted for in the final outcome measurement. None of the studies adjusted for these factors. Moreover, most surrogate markers are highly dependent on the ultrasonographist(s) and/or interpreter(s) of the images. Hence, blinding is required but this was not described in 16 out of the 30 studies. Finally, many studies included a very limited number of patients. Only three studies included ≥50 participants for both groups.

Heterogeneity of studies

Substantial heterogeneity existed between studies. In addition to the methodological limitations, the study populations varied in ‘time since KD’, ‘number of CAA-positive patients’, ‘percentage of IVIG-treated patients’, ‘gender distribution’, ‘age’, ‘ethnicity’ and ‘CAA-criteria’. When exploring heterogeneity by analyzing the first three variables, we could only find a significant covariate for the meta-analyses on SI. It was however, difficult to define some of these variables: the percentage of CAA-positive patients does not necessarily correlate to the severity of the aneurysms; some studies only included patients with transient dilations, while others included patients with severe or persistent aneurysms.

Endothelial (dys)function

FMD in correlation with CVD risk has been researched extensively. Ras et al. found a CVD risk ratio of 0.9 per 1% higher FMD in a systematic review in adults. In children,
a significantly lower FMD has been found in sub-populations with an increased cardiovascular risk such as familial hypercholesterolemia\textsuperscript{64}.

FMD is an endothelium-dependent marker, which is mediated by the release of nitric oxide (NO)\textsuperscript{44}. In contrast to FMD, NMD is an endothelium-independent marker, thought to reflect smooth muscle (dys)function. It has shown to be increased in diabetes mellitus and hypertension and is suggested to be a marker of the grade of cardiovascular risk\textsuperscript{46}. NMD was not increased in any of the studies in this review, suggesting that the patients are at-risk but the endothelial dysfunction is at an early stage when smooth muscle function is not (yet) affected.

PAT is thought to correlate with coronary endothelial dysfunction. Studies in adults and children have shown a correlation between a lower PAT and coronary atherosclerosis and cardiovascular events or risk factors\textsuperscript{47,48}. An earlier, large cohort study did not show correlation between FMD and PAT, indicating that they might reflect distinct aspects of endothelial function and possibly explaining the difference in PAT and FMD in our review\textsuperscript{49}.

### Arterial stiffness

Aortic PWV is a known predictor of cardiovascular events\textsuperscript{50}. In contrast, studies included in this review measured brachial-radial PWV or baPWV. Earlier studies found a significant correlation between baPWV and cardiovascular events or risk factors\textsuperscript{47,48}. Brachioradial PWV is less common in use and to our knowledge, no large studies looking at the association between brachioradial PWV and cardiovascular events have been performed.

SI has shown to be increased in children with obesity and in adults after myocardial infarction, although no large studies have investigated the exact correlation between CVD event and SI\textsuperscript{51,54}.

### Carotid IMT

CIMT is a validated measure of cardiovascular risk. Lorenz et al. found a hazard ratio (HR) of 1.15 for myocardial infarction (MI) and 1.18 for stroke with every 0.1 mm increase in cIMT in their systematic review\textsuperscript{6}. In addition, they showed that people <50 years of age are at higher relative risk with increasing cIMT compared to people >50 years\textsuperscript{53,54}. Brachioradial PWV is less common in use and to our knowledge, no large studies looking at the association between brachioradial PWV and cardiovascular events have been performed.

Although cIMT is validated, it is important to realize that a distance of 0.5 mm is a large standard deviation by default, hence not suitable for research in small groups. In our review, 13 out of the 15 studies measuring cIMT included less than 50 participants per group.

### Cardiovascular disease risk

Even though most surrogate markers for CVD risk showed a significant difference between one of the KD-groups and controls in most studies, the pathophysiological mechanism behind these changes following KD is still unclear. In fact, post-mortem studies have failed to show atherosclerotic changes, even in affected coronary arteries\textsuperscript{57}. In this post-mortem study, active remodeling of the coronary arterial wall could be identified years after the acute stage of the disease, potentially indicating that a distinct cardiovascular process, other than atherosclerosis, may be held responsible for an increased CVD risk following the early period of acute vascular inflammation in KD. Prolonged (low-grade) inflammation as suggested by the presence of increased levels of inflammatory markers such as (high-sensitivity) CRP are believed to be associated with the occurrence of cardiovascular events in adults\textsuperscript{57,58}. However, controversy exists as to whether patient with KD have a continued low-grade inflammation years after the disease\textsuperscript{57,58,33,37}.

A pathophysiological mechanism responsible for the changes in the vasculature in KD, both in the coronary and the peripheral arteries, has yet to be elucidated. Whether persistent low-grade inflammation or genetic factors may play a role in this “KD-vasculopathy” and in remodelling of the arterial wall is as yet unclear.

### Limitations

Some limitations of our study have to be mentioned. First, we found substantial heterogeneity between studies. Because of this heterogeneity, we could not pool most of the results from the original studies. Hence, conclusions can only be drawn from a summary of these studies without a statistical finding.

Although we tried to find the source of heterogeneity by performing meta-regression analyses, we could not find factors for all surrogate markers. For both cIMT and SI, we could include less than 10 studies in the meta-regression analyses; it is questionable whether such numbers are large enough because of a lack of power.

For this review we considered patients who ever had CAA as CAA-positive. However, CAA range from small to giant, reflecting the severity of the original vasculitis and it may thus not be appropriate to combine all CAA-positive patients into one group.

None of the included studies reported on long-term longitudinal data as they were all cross-sectional or very short-term cohort studies. Long-term follow-up is necessary to investigate the course of the surrogate markers over time as well as the natural course of the disease and to predict CVD risk at a later age.

### Conclusion

This systematic review and meta-analyses suggests that surrogate markers for CVD risk in patients after KD are increased in CAA-positive but not in CAA-negative patients.
The results have to be interpreted with care due to methodological limitations and high heterogeneity between studies which prevents the possibility of data pooling. However, these findings might indicate that CAA-positive patients should be monitored and counselled for CVD in later life. Long-term follow-up of former KD patients is needed to confirm our results.

Acknowledgements

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References


## Chapter 3

### Peripheral Endothelial (Dys)function, Arterial Stiffness and Carotid Intima-Media Thickness

### Supporting Checklist (S1 Supporting Checklist)

<table>
<thead>
<tr>
<th>Section/topic</th>
<th>Checklist item</th>
<th>Reported on page #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title</strong></td>
<td>Identify the report as a systematic review, meta-analysis, or both.</td>
<td>1</td>
</tr>
<tr>
<td><strong>Abstract</strong></td>
<td>Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.</td>
<td>2</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td>Describe the rationale for the review in the context of what is already known.</td>
<td>3</td>
</tr>
<tr>
<td><strong>Objectives</strong></td>
<td>Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).</td>
<td>3</td>
</tr>
<tr>
<td><strong>Methods</strong></td>
<td>Identify a review protocol, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.</td>
<td>5</td>
</tr>
<tr>
<td><strong>Eligibility criteria</strong></td>
<td>Specify study characteristics (e.g., PICOS, language, publication status) used as criteria for eligibility, giving rationale.</td>
<td>5</td>
</tr>
<tr>
<td><strong>Information sources</strong></td>
<td>Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies in the search and data last searched).</td>
<td>5</td>
</tr>
<tr>
<td><strong>Search</strong></td>
<td>Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.</td>
<td>Supplemental file</td>
</tr>
<tr>
<td><strong>Study selection</strong></td>
<td>State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).</td>
<td>5</td>
</tr>
<tr>
<td><strong>Data collection process</strong></td>
<td>Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.</td>
<td>5</td>
</tr>
<tr>
<td><strong>Data items</strong></td>
<td>List all data items for which data were sought (e.g., PICOS, funding sources) and any assumptions and implications made.</td>
<td>5</td>
</tr>
<tr>
<td><strong>Risk of bias in individual studies</strong></td>
<td>Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.</td>
<td>5</td>
</tr>
<tr>
<td><strong>Summary measures</strong></td>
<td>State the principal summary measures (e.g., risk ratio, difference in means).</td>
<td>6</td>
</tr>
<tr>
<td><strong>Risk of bias across studies</strong></td>
<td>Describe methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I$^2$ for each meta-analysis).</td>
<td>6</td>
</tr>
<tr>
<td><strong>Additional analyses</strong></td>
<td>Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).</td>
<td>6</td>
</tr>
<tr>
<td><strong>Risk of bias across studies</strong></td>
<td>Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were prespecified.</td>
<td>6</td>
</tr>
<tr>
<td><strong>Results</strong></td>
<td>Give number of studies searched, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.</td>
<td>8 + Figure 1: flow diagram</td>
</tr>
<tr>
<td><strong>Study characteristics</strong></td>
<td>For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.</td>
<td>Table 1: Study characteristics.</td>
</tr>
<tr>
<td><strong>Risk of bias within studies</strong></td>
<td>Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).</td>
<td>Figure 2: quality of studies and 2a table: quality assessment per study</td>
</tr>
<tr>
<td><strong>Synthesis of results</strong></td>
<td>Briefly summarize findings, including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).</td>
<td>Table 2: summary of findings</td>
</tr>
<tr>
<td><strong>Risk of bias across studies</strong></td>
<td>21 Present results of each meta-analysis done, including confidence intervals and measures of consistency.</td>
<td>Figure 3-5</td>
</tr>
<tr>
<td><strong>Additional analyses</strong></td>
<td>23 Present results of any analysis of bias across studies (see item 19).</td>
<td>8, 12, 17-21</td>
</tr>
<tr>
<td><strong>Discussion</strong></td>
<td>Summarize the main findings, including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).</td>
<td>22</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>24 Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).</td>
<td>25</td>
</tr>
<tr>
<td><strong>Conclusions</strong></td>
<td>Provide a general interpretation of the results in the context of other evidence, and implications for future research.</td>
<td>25</td>
</tr>
<tr>
<td><strong>Funding</strong></td>
<td>27 Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.</td>
<td>Submission</td>
</tr>
</tbody>
</table>

### Supporting File (S1 File)

**Electronic Medline Search Strategy**

- Continent.
- Environment.
- Gene.
- Human.
- In Animals.
- In Vitro.
- Interventions.
- Language.
- Medline.
- Meta Analysis.
- Outcomes.
- Phlebotomy.
- Pneumonia.
- Rehabilitation.
- Randomized Controlled Trial.
- Respiratory System.
- Special Topics.
- Systematic Review.
- Therapeutic Procedure.
- Therapeutics.

**Medline electronic search strategy**

- **Cardiac Intima-Media Thickness** (MeSH) OR intima media thickness (tab) OR IMT (tab) OR intimal thick (tab) OR myointimal thick* (tab) OR "Vascular Stiffness" (MeSH) OR stiffness OR stiffening OR endothelial dysfunction (tab) OR endothelial function (tab) OR flow mediated dilat* (tab) OR flow-mediated dilat* (tab) OR FMD (tab) OR anery dilat* (tab) OR anery ativity dilat* (tab) OR "Compliance" (MeSH) OR Densibility (tab) OR "Pulse Wave Analysis" (MeSH) OR Pulse wave analy* (tab) OR pulse wave velocity (tab) OR PWV (tab) OR "Atherosclerosis" (MeSH) OR atherosclerosis (tab) OR athero lesion (tab) OR vascular ultrasound (tab) OR: vascular elasticity (tab) OR peripheral arterial tonometry (tab) OR endo pat (tab) OR cardiovascular risk (tab) AND (Mucocutaneous Lupus Nephritis Syndrome) (MeSH) OR Kawasaki (tab)
### SUPPORTING TABLE 1 (S1 TABLE) QUALITY ASSESSMENT CRITERIA

<table>
<thead>
<tr>
<th>Selection</th>
<th>Comparability</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of controls</td>
<td>Identification of controls</td>
<td>Correction for factors in outcome</td>
</tr>
<tr>
<td>No description</td>
<td>No description</td>
<td>No clear description</td>
</tr>
</tbody>
</table>

#### Adequate (++)
- Full eligibility criteria for all eligible cases with KD selected.
- Or all cases with KD in a defined hospital or clinic, group of hospitals, health organization, or an approp rate sample of those cases (e.g. random sample).
- Community-based controls, hospital-based controls, clearly stated from where.
- No history of premature atherosclerotic disease, or hypermetabolism, hypercholesterolemia or other factors that might affect vascular function.
- Identification of known factors to influence the surrogate markers including age, gender, blood pressure and BMI.
- Clear description of cIMT, protocol, site, machine, PWV, site, method, machine PA, site, method and machine SI, site, method of calculation and machine.

#### Moderate (+)
- Full eligibility criteria for all eligible cases with KD selected.
- Or all cases with KD in a defined hospital or clinic, group of hospitals, health organization, or an approp rate sample of those cases (e.g. random sample).
- Community-based controls, hospital-based controls, clearly stated from where.
- No history of premature atherosclerotic disease, or hypermetabolism, hypercholesterolemia or other factors that might affect vascular function.
- Identification of known factors to influence the surrogate markers including age, gender, blood pressure and BMI.
- Clear description of cIMT, protocol, site, machine, PWV, site, method, machine PA, site, method and machine SI, site, method of calculation and machine.

#### Incomplete (-)
- Full eligibility criteria for all eligible cases with KD selected.
- Or all cases with KD in a defined hospital or clinic, group of hospitals, health organization, or an approp rate sample of those cases (e.g. random sample).
- Community-based controls, hospital-based controls, clearly stated from where.
- No history of premature atherosclerotic disease, or hypermetabolism, hypercholesterolemia or other factors that might affect vascular function.
- Identification of known factors to influence the surrogate markers including age, gender, blood pressure and BMI.
- Clear description of cIMT, protocol, site, machine, PWV, site, method, machine PA, site, method and machine SI, site, method of calculation and machine.

### SUPPORTING TABLE 2 (S2 TABLE) QUALITY ASSESSMENT PER STUDY

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient group</th>
<th>Representativeness</th>
<th>Selection of controls</th>
<th>Definition of controls</th>
<th>Identification of controls</th>
<th>Comparability</th>
<th>Correction</th>
<th>Description</th>
<th>Blinding</th>
<th>Outcome measured in a blinded manner</th>
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<tr>
<td>Dhillon, 1996</td>
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<td>++</td>
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<tr>
<td>Silva, 2001</td>
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<td>+</td>
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<td>Noto, 2009</td>
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<td>+</td>
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<td>++</td>
<td>++</td>
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<td>Deng, 2002</td>
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<td>4/1/4</td>
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</table>
Carotid intima-media thickness in patients with a history of Kawasaki disease


* Authors contributed equally

Circulation journal 2015; 79: 2682-2687
Chapter 4 Carotid intima-media thickness in patients with a history of Kawasaki disease

Background: Kawasaki disease (KD) is an acute pediatric vasculitis with coronary artery aneurysms (CAA) as its main complication. Concerns have been raised regarding the possibility of a predisposition of KD to premature cardiovascular disease (CVD) risk later in life. Our aim was to assess carotid intima-media thickness (cIMT), a surrogate marker of CVD risk, in patients with a history of KD compared to unaffected controls.

Methods and results: B-mode ultrasound carotid intima-media thickness (cIMT) measurements were performed in 168 patients with a history of KD, and 82 controls. Seven patients were excluded because of incomplete cIMT assessments.

Mean cIMT (±SD) was increased in patients with KD compared to controls (0.378±0.030 mm versus 0.360±0.027 mm, respectively; P adjusted<0.0001). If cIMT of CAA-negative patients and controls were plotted against age, increased cIMT was only apparent at young age. In patients with CAA, increased cIMT was observed over the entire age range.

Conclusion: Our findings show that arterial wall thickening is more apparent in patients with a history of KD as compared to controls. In CAA-negative patients, cIMT is indistinguishable from controls at older age, whereas an increased cIMT is observed at any age in patients with CAA, suggesting a more general and severe impact of KD on the arterial wall.

Introduction
Kawasaki disease (KD) is an acute systemic vasculitis that predominantly occurs in children less than five years of age. The disease is thought to be caused by an infectious agent in genetically predisposed children. Coronary artery aneurysms (CAA) develop in 15 to 25% of untreated patients and may lead to myocardial ischemia, infarction and, sudden death. Although treatment with high-dose intravenous immunoglobulins (IVIG) has reduced this risk to less than 10%, KD is the leading cause of acquired heart disease in developed countries.

The disease is self-limiting and only rarely recurs, but there has been ongoing concern that patients both with and without coronary artery involvement may have a predisposition to endothelial damage and premature atherosclerotic disease in adulthood. Thickening of the coronary arterial wall has been shown in persisting and regressed dilatations, but also in always-normal coronary segments. Since the first case of KD was reported in 1967, patients who have recovered will now be middle-aged or younger, and therefore the follow-up of these patients has not been long enough to establish the natural history of the disease.

To determine if KD is a risk factor for the future development of cardiovascular disease (CVD), several studies reported on the carotid intima-media thickness (cIMT) of patients with a history of KD. A thickened intima-media complex can be a result of atherosclerosis but can also be caused by other processes involving injury and inflammation. As assessed by B-mode ultrasound, it is currently the best-validated non-invasive surrogate marker for cardiovascular risk available. An increased cIMT has been reported in former KD patients both with and without CAA in the past, although these findings have not been confirmed by other studies.

We hypothesized that, due to their history of a systemic vasculitis, patients with KD have an increased risk of CVD. Therefore, to determine CVD risk in patients with a history of KD, B-mode ultrasound cIMT-measurements – as a surrogate marker of CVD risk – in KD and unaffected control subjects were performed.

Methods
Participants
The study was conducted between January 2008 and September 2013 at the Emma Children’s hospital, a tertiary referral centre. Children aged 7 to 20 years with a history of KD were recruited consecutively during follow-up as outpatients. The diagnosis of KD was based on criteria from the American Heart Association. Patients diagnosed as having KD within six months of the study were excluded to minimize the potential confounding influence of the (sub)acute inflammation. If multiple IMT-measurements had been performed, the last measurement was included in the study.
Unaffected siblings of children with KD and other unafflicted subjects (family from the staff of our hospital) without a history of KD were eligible for the control cohort if they were in the same age range and did not take any cardiovascular medication. All subjects and/or their parents gave informed consent as approved by the institution’s Research Ethics Board.

Study protocol
A medical (family) history was obtained from all participants, and body height, weight and, blood pressure was measured. A non-invasive measurement of the carotid IMT was performed as described below. The mean arterial pressure (MAP) was calculated using the following formula: (systolic blood pressure + (2*diastolic blood pressure))/3.

Using data of the fifth Dutch growth study performed in 2009 in 20,867 children in The Netherlands, standard deviation scores (SDS) for body mass index (BMI) were calculated based on the age and gender of each participant.

The medical records of the patients with KD were reviewed retrospectively to collect the following clinical details: age at disease onset, time interval between disease onset and time of study, treatment with IVIG, aspirin and/or steroids, and the presence of CAA. The coronary arteries had been evaluated by two-dimensional echocardiography. We defined CAA by worst-ever z-scores: CA-dimensions as standard deviation units normalized for basal surface area. We choose to define the CAA by their worst-ever z-scores because even when the lumen of a previously affected coronary artery has returned to its normal size, the artery can still be damaged and thus the initial systemic vasculitis was clearly more severe when compared to children with a normal size who never had any enlargement at all. CAA was defined as a coronary z score ≥ 2.5, a giant aneurysm was defined as a z-score of ≥ 10 or a diameter of ≥ 8 mm. In the patients with KD, a venous blood sample was taken after an overnight fast for measurement of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B, lipoprotein(a) and, apolipoprotein E genotype. LDL-cholesterol was calculated using the Friedewald formula.

Carotid IMT measurements
Two experienced and certified ultrasonographers scanned the subjects. An Acuson Sequoia 512 ultrasound instrument equipped with an 8L5 8-5 MHz linear array vascular transducer was used. All B-mode ultrasound scans were done according to a standardized protocol. Of every subject the right and left common carotid, carotid bulb and internal carotid arterial segments were visualized. One image of each segment was saved as 2x2cm high resolution DICOM still. Image analysis was done off-line in a core lab, of which 20 images were analysed twice to assess intrarater reliability. The intraclass correlation coefficient was 0.92 (95% Confidence Interval [95% CI], 0.75-0.97) for the mean cIMT.

One image analyst performed all cIMT measurements blinded for the patient’s case status and risk factor levels. The per subject mean combined cIMT was calculated as follows: (mean of the left and right common carotid arteries + the mean of the left and right carotid bulb + the mean of the left and right internal carotid far wall segments)/3. For subjects in whom the scan of one of the segments had failed, the measurement of the same segment of the opposite carotid artery was taken as the mean of both carotid arteries. If both left- and right-side values were unavailable, the IMT was considered missing for that segment, and in that case the mean combined cIMT was also considered missing.

Statistical analysis
We evaluated differences in demographics between patients with KD and controls by linear or logistic regression analysis. Differences in cIMT between patients with KD and controls were evaluated using linear regression analyses. We adjusted for the following potential confounders: age, gender, BMI SDS, mean arterial pressure, family history. In addition, we performed stepwise backward elimination. Furthermore, in the group of KD patients we evaluated if IVIG treatment, IVIG resistance, total cholesterol, LDL-cholesterol and triglycerides were associated with cIMT by linear regression analysis.

An equation for difference in cIMT (ΔcIMT) was derived by subtracting the equation for patients with KD (if GROUP=1), that is, IMT=β1AGE+β2+β3AGE, from the equation for the unaffected controls (if GROUP=0), that is, IMT=β1AGE. This calculation resulted in ΔcIMT=β2+β3AGE. Betas and standard errors were derived from the output of a linear regression analysis for the whole group. Linear and logistic regression analyses were performed using the generalized estimating equation method in the SAS procedure GENMOD to account for correlations within families. The exchangeable correlation structure was used for these models. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using SAS release version 9.2 (SAS Institute, Cary, NC) and SPSS version 20.0 software (SPSS Inc, Chicago, IL).

Results
In total, 168 former patients with KD and 82 controls subjects were enrolled. The control group consisted of 74 unaffected siblings and eight family members from the hospital staff. Seven patients were excluded because of missing cIMT segments. Demographic characteristics of the remaining 161 patients with KD and the 82 controls were similar with respect to mean age, sex distribution, mean BMI SDS and MAP (Table 1). Clinical
TABLE 1 Demographic data of former KD patients and controls, and clinical and laboratory data of the patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=161</td>
<td>n=82</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.0 ± 3.0</td>
<td>12.3 ± 3.4</td>
</tr>
<tr>
<td>Subgroup 7-10 years</td>
<td>73 (45%)</td>
<td>34 (47%)</td>
</tr>
<tr>
<td>Subgroup 11-15 years</td>
<td>54 (34%)</td>
<td>30 (37%)</td>
</tr>
<tr>
<td>Subgroup 16-20 years</td>
<td>34 (21%)</td>
<td>18 (22%)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>100 (62)</td>
<td>44 (54)</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>154 ± 0.18</td>
<td>155 ± 0.17</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>40 ± 10</td>
<td>47 ± 19</td>
</tr>
<tr>
<td>BMI SD score</td>
<td>0.47 ± 1.2</td>
<td>0.26 ± 1.1</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>80.1 ± 0.71</td>
<td>81.4 ± 0.88</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>20 (12.4)</td>
<td>13 (15.9)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>131 (81.4%)</td>
<td>69 (84.1%)</td>
</tr>
<tr>
<td>African American</td>
<td>6 (3.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (1.9%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>12 (7.5%)</td>
<td>8 (9.8%)</td>
</tr>
<tr>
<td>Other</td>
<td>9 (5.6%)</td>
<td>4 (3.7%)</td>
</tr>
<tr>
<td>Age at disease onset (years)</td>
<td>3.1 ± 2.5</td>
<td>-</td>
</tr>
<tr>
<td>Interval between disease onset and cIMT measurement (years)</td>
<td>8.0 (6.1-10.9)</td>
<td>-</td>
</tr>
<tr>
<td>Treatment with IVIG, n (%)</td>
<td>145 (90)</td>
<td>-</td>
</tr>
</tbody>
</table>

Lipid profile:
<table>
<thead>
<tr>
<th>Patients</th>
<th>Reference values for age</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=161</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L), 7-12 yrs</td>
<td>4.17 ± 0.59</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L), 13-20 yrs</td>
<td>3.95 ± 0.72</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L), 7-12 yrs</td>
<td>2.30 ± 0.54</td>
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<tr>
<td>LDL-cholesterol (mmol/L), 13-20 yrs</td>
<td>2.20 ± 0.60</td>
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<tr>
<td>HDL-cholesterol (mmol/L), 7-12 yrs</td>
<td>1.51 ± 0.40</td>
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<tr>
<td>HDL-cholesterol (mmol/L), 13-20 yrs</td>
<td>1.30 ± 0.29</td>
</tr>
<tr>
<td>Triglycerides (mmol/L), 7-12 yrs</td>
<td>0.71 (0.53-0.93)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L), 13-20 yrs</td>
<td>0.70 (0.54-1.05)</td>
</tr>
<tr>
<td>Apolipoprotein A4 genotype, n (%)</td>
<td>41 (25.5)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation unless otherwise noted.

a. First degree relative with cardiovascular risk factor or disease.

b. Of Turkish, Moroccan or Indo- Surinamese descent.
c. Median (interquartile range).
d. To convert mmol/L to mg/dl: for total, HDL and LDL multiply by 38.61, for triglycerides multiply by 88.50.
e. Normal Dutch reference values per age category, 2.5-97.5 percentile (28).
f. MAP: mean arterial pressure; n: number; BMI: body mass index; SD: standard deviation; IVIG: intravenous immunoglobulin; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

Discussion

This study shows that children with a history of KD have an increased cIMT compared to unaffected controls. Plotting the difference in cIMT between patients and controls against age indicated that the observed difference in cIMT diminished with increasing age and disappeared in young adulthood in patients without CAA. In contrast, in children with CAA during acute KD – being either transient or persistent – the difference in cIMT and laboratory data of patients with a history of KD are shown in Table 1. Median (interquartile range) onset of KD disease was 3.1 (1.2-5.3) and 145 (90%) patients were treated with IVIG.

Based on their worst-ever coronary artery z-score, 119 (75%) had no CAA (z-score <2.5 during the (sub)acute phase) and 42 patients had CAA (31 with a z-score of 2.5-10; and 11 with a z-score ≥ 10 or a diameter of ≥ 8 mm). Of all patients, 145 (90%) had been treated with IVIG. Of these 145 patients, 27 (18.6%) were IVIG resistant and received another dose of IVIG. Baseline characteristics of the CAA-negative and CAA-positive group are shown in supplemental Table 1.

Carotid IMT

The mean combined cIMT (±SD) was increased in patients with KD if compared to unaffected controls (0.378±0.030 mm vs. 0.360±0.027 mm; P<0.0001). After adjustment for age, gender, BMI, MAP, family history and family relations, the difference remained statistically significant (P<0.0001). This result did not change when BMI, MAP and family history were removed using stepwise backward elimination. Table 2 shows the mean cIMT, adjusted for age, gender and family relations, for controls and separate subgroups of patients with KD.

In univariate analysis, IVIG treatment, IVIG resistance, total cholesterol, LDL-cholesterol and triglycerides were not significantly associated with mean cIMT.

For all subjects in the patient group, KD occurred around the age of 3 years. Because the age of the cIMT measurement varied considerably (range: 7–20 years), we could explore the association between the difference in cIMT between KD patients and controls (ΔcIMT), and the time since KD onset. For this purpose, we plotted ΔcIMT against age at cIMT measurement (Figure 1A).

Based on their worst-ever z-score, plots were also created for subgroups without CAA (Figure 1B) and with CAA (Figure 1C). In CAA-negative patients, ΔcIMT diminished with age at time of the cIMT measurement, and disappeared, as age increased. In contrast, in patients with CAA a difference between patients and controls in cIMT remained present at all ages. When we excluded patients with giant aneurysms from the statistical analyses, these results on outcome remained unchanged.
PART I

was observed at all ages and remained significantly different from unaffected controls at all ages.

Studies evaluating the cIMT in patients with a history of KD are limited, and the studies that have been performed mainly included small numbers of patients and have produced conflicting results. We have recently published a systematic review and meta-analysis on CVD risk in patients with KD, including a total of 15 cIMT studies. Some of these studies reported no difference between KD patients and controls, whereas others found an increased cIMT in CAA-positive patients or also in CAA-negative patients. Quality assessment showed that all these studies had some significant methodological limitations. Mean cIMT in the whole KD group and the CAA-positive group did not differ significantly with controls upon statistical meta-analysis, although there was a trend toward a thicker cIMT in patients (0.01 mm, 95% CI 0.00-0.02 and 0.01 mm, 95% CI 0.00-0.03 mm, respectively).

In contrast to these findings, the results of the present study show that subjects with a history of KD have on average a significantly greater mean cIMT compared to unaffected controls. However, the difference in cIMT diminished when analysed at different ages in the CAA-negative but not in CAA-positive patients. When analysing the cIMT data in this way, i.e. plotted against age of measurements, our findings may explain at least in part the conflicting data of many of the prior studies that have used different patient groups and variable ages at analysis. Our study would indicate that the majority of KD patients tends to ‘normalize’ over time to eventually fall in the ranges of normal unaffected controls. However, our study similarly indicates that the cIMT of CAA-positive patients remains abnormal at all ages and can be distinguished as long as we were able to assess cIMT in our cohort of KD patients.

KD is a vasculitis that predominantly occurs in very young children. The median age at KD onset in our study cohort was 3.1 years. Therefore, the mean time interval from disease onset to participation in this study increases with age. Although it is not

TABLE 2

Mean carotid IMT of controls and subgroups of patients with a history of Kawasaki disease based on their coronary artery status

<table>
<thead>
<tr>
<th></th>
<th>Total (%)</th>
<th>Mean cIMT (mm) b</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>82 (100)</td>
<td>0.360 ± 0.003</td>
<td>Reference</td>
</tr>
<tr>
<td>No enlargement (z-score &lt;2.5)</td>
<td>119 (73.9)</td>
<td>0.376 ± 0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAA (z-score 2.5-10)</td>
<td>31 (19.3)</td>
<td>0.373 ± 0.006</td>
<td>0.064</td>
</tr>
<tr>
<td>Giant CAA (z-score ≥10)</td>
<td>11 (6.8)</td>
<td>0.412 ± 0.010</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a. Based on worst-ever z-score during acute phase of either left coronary artery, right coronary artery or left anterior descending artery
b. IMT-mean adjusted for age and gender (mean ± standard error)
cIMT: carotid intima-media thickness; CAA: coronary artery aneurysms

FIGURE 1

The difference in mean combined cIMT and 95% CI between patients with a history of KD and unaffected controls

The difference in mean combined carotid intima-media thickness (cIMT) and 95% CI between patients with a history of Kawasaki disease and unaffected subjects plotted against age, taking family relations into account
A: the difference between the whole group of patients and unaffected subjects (n=243); B: the difference between the CAA-negative patients and unaffected subjects (n=201); C: the difference between CAA-positive patients and unaffected subjects (n=124).

Mean = thick line; 95%CI = dashed lines.
clear what pathophysiologic process results in the increased cIMT of CAA-positive patients as well as in the initially increased cIMT of CAA-negative patients, our findings suggest that this process represents a form of vasculopathy that may be different from premature atherosclerosis. If KD would result into premature atherosclerosis, ΔIMT is to be expected to further increase at older age, whereas cIMT of CAA-negative patients has become indistinguishable from controls in the older children instead.

The assumption that it may be a vasculopathy would be in line with an earlier study looking at the pathology of arteries in KD patients who died of myocardial infarction or heart failure 2 to 12 years after the onset of Kawasaki disease. A markedly thickened intima was found in the aneurysms that became stenotic and showed active remodelling of the arterial lesions many years after the disease. This process of remodelling was accompanied by the expression of multiple vascular growth factors including PDGF and TGF-β1 but no fatty streaks or accumulation of macrophages as is seen in (premature) atherosclerosis. Although we may not extrapolate these findings to other vascular structures including the carotid artery, the finding that TGF-β is involved in arterial remodelling, could explain the non-progressive nature of the cIMT in CAA-negative patients up to 15 years after the disease. Such would not be in line with a process of premature atherosclerosis and supports the possibility of a different form of remodelling, provisionally designated as KD-vasculopathy.

In CAA-positive patients this pathway might continue to be active resulting in a continuing increase of the cIMT. We have previously reported on the presence of vascular growth factors, TGF-β and their genetic association with KD. The precise nature of the vasculopathy that might result in a thickened carotid intima-media in KD patients has not yet been determined.

The American Heart Association recommends life-long follow-up for both children with and without CAA to assess CVD risk. Controversy exists on whether there is a need for this in children who never or only transiently experienced CAA. The uncertainty about a possible increased cardiovascular risk based on the systemic vasculitis in the past feeds a big part of this discussion. Our study results indicate that a life-long follow-up of children without CAA might not be necessary, although additional factors may be in play. For that reason longitudinal studies have to be performed to define the real risk for CVD using the same methodology to decide about the follow-up of these children.

A major strength of the present study was the large study group and the consistent use of a standardized carotid imaging and image analysis protocol. Ultrasound measurements of all participants were obtained by two experienced sonographers, and one image analyst blinded for case or CAA status read all the images.

Some methodological aspects of our study merit discussion. First, we have only included one IMT measurement of each patient. The cIMT progression estimates were therefore based on cross-sectional data. Long-term follow-up studies are warranted to assess the ‘real’ course of cIMT change over time in the same KD patients. Second, patients were stratified based on their ‘worst-ever’ z-score. Because the study was performed in a tertiary referral centre, pediatric cardiologist in other centres than ours generated many of the early echocardiograms. This might have caused misclassification of patients in the stratification for CAA subgroups. Third, although patients were included in a consecutive order at the outpatient clinic, the study population contains a high percentage of patients with CAA, as explained by referral bias with the more severe cases at our tertiary centre. This may have overestimated the mean IMT of the total KD group.

Conclusion
Our study of cIMT in KD patients shows that the signs of early arterial wall changes are more apparent in patients with a history of KD, in particular in those with CAA. When plotting the difference in cIMT between patients and controls in patients without CAA, cIMT became indistinguishable from controls with age. In CAA-positive patients an increased cIMT was observed at any age, the latter demonstrating a more severe impact of KD on the arterial wall. Although longitudinal data are missing, this result suggests that follow-up seems justified in CAA-positive patients, but may not be necessary in CAA-negative patients.
CHAPTER 4 CAROTID INTIMA-MEDIA THICKNESS IN PATIENTS WITH A HISTORY OF KAWASAKI DISEASE

References


### Supporting information

Supporting information applicable for Chapter 4.

#### SUPPLEMENTAL TABLE 1  Baseline characteristics CAA-positive and -negative subgroups according to worst-ever z-score

<table>
<thead>
<tr>
<th></th>
<th>CAA-negative n=119</th>
<th>P-value vs controls</th>
<th>CAA-positive N=42</th>
<th>P-value vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>11.7 ± 3.1</td>
<td>0.207</td>
<td>13.0 ± 3.6</td>
<td>0.245</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>65 (55)</td>
<td>1.000</td>
<td>35 (83)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>152 ± 17</td>
<td>0.243</td>
<td>158 ± 19</td>
<td>0.444</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>44 ± 10</td>
<td>0.209</td>
<td>50 ± 18</td>
<td>0.205</td>
</tr>
<tr>
<td>BMI SD score</td>
<td>0.40 ± 1.10</td>
<td>0.380</td>
<td>0.65 ± 1.26</td>
<td>0.083</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>78.6 ± 6.2</td>
<td>0.017†</td>
<td>83.9 ± 7.9</td>
<td>0.139</td>
</tr>
<tr>
<td>Age at disease onset (years)</td>
<td>3.4 (1.8-5.6)</td>
<td>7.5 (5.4-10.1)</td>
<td>9.6 (7.5-12.2)</td>
<td></td>
</tr>
<tr>
<td>Interval between disease onset and cIMT measurement (years)</td>
<td>3.4 (1.8-5.6)</td>
<td>7.5 (5.4-10.1)</td>
<td>9.6 (7.5-12.2)</td>
<td></td>
</tr>
<tr>
<td>Treatment with IVIG, n (%)</td>
<td>107 (90)</td>
<td>38 (90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Timing of IVIG, days‡</td>
<td>7 (6-9)</td>
<td>8 (6-12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.13 ± 0.63</td>
<td>3.00 ± 0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.29 ± 0.56</td>
<td>2.09 ± 0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.46 ± 0.37</td>
<td>1.40 ± 0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.70 (0.48-0.94)</td>
<td>0.78 (0.59-0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein E4 genotype, n (%)</td>
<td>30 (25.2)</td>
<td>11 (26.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation unless otherwise noted. *There were more boys in the CAA-positive group as male sex is a risk factor for CAA. This was corrected for in final analysis.

†MAP in the CAA-negative group was lower than in controls, but MAP was removed from the multivariable regression analyses by stepwise backward elimination. ‡Median (interquartile range). MAP: mean arterial pressure; n: number; BMI: body mass index; SD: standard deviation; IVIG: intravenous immunoglobulin; LDL: low-density lipoprotein; HDL: high-density lipoprotein.
Extracardial vasculopathy after Kawasaki disease: a long-term follow-up study


JAHA, 2016; 5: e003414
Chapter 5 Extracardial vasculopathy after Kawasaki disease: a long-term follow-up study

Background: Kawasaki disease (KD) is a pediatric vasculitis with coronary artery aneurysms (CAA) as major complication. Controversy exists about cardiovascular risk later in life. The aim of our study was to evaluate whether KD patients are at increased risk as assessed by carotid intima-media thickness (cIMT).

Methods and results: Over 15 years we measured cIMT by B-mode ultrasonography in KD patients during follow-up, and in unaffected controls (mostly sibs). A multilevel, repeated-measures, linear-mixed-effects model was used to evaluate the association between KD and cIMT.

A total of 319 patients with 528 measurements were compared to 150 controls. In KD patients the mean cIMT was increased when compared to controls (0.375 mm [95% CI 0.372-0.378 mm] vs 0.363 mm [95% CI 0.358-0.368 mm]; p < 0.001). Furthermore, CAA-negative patients had a mean cIMT of 0.373 mm (p < 0.01 compared to controls), patients with small-medium CAA 0.374 mm (p < 0.05 compared to controls) and patients with giant CAA 0.381 mm (p < 0.01 compared to controls). As compared to controls, CAA-negative subjects started with an increased cIMT (+0.0193 ± 0.0053 mm, p < 0.001), but showed slower progression (-0.0014 ± 0.0006 mm/year, p = 0.012). Patients with giant CAA showed a trend towards increased cIMT progression (+0.0013 ± 0.0007 mm/year, p = 0.058).

Conclusion: We observed a positive correlation between cIMT and KD severity of coronary arteritis at the acute stage. Although initially increased, the cIMT in CAA-negative patients normalized at a later age. In contrast, patients with a history of KD complicated by giant CAA showed a trend towards a persistently increased cIMT. These patients may need cardiovascular counseling and follow-up beyond the heart.

Introduction
Kawasaki disease (KD) is an acute systemic vasculitis, predominantly occurring in children less than five years of age. The main complication of this disease is the development of coronary artery aneurysms (CAA). CAA develop in 15 to 25% of untreated patients.

KD is the leading cause of acquired heart disease in developed countries. Standard treatment consists of a single administration of high-dose intravenous immunoglobulins (IVIG) and oral aspirin for 6-8 weeks. This has been shown to reduce the risk of CAA to less than 10% when treated within 10 days. Both the etiology and pathophysiology of KD, as well as the working mechanism of IVIG have remained unclear to date.

It has been hypothesized that KD represents a systemic vasculitis that, apart from the absence or presence of CAA, results in increased cardiovascular disease (CVD) risk at a later age. Although controversial, this hypothesis was supported by abnormal myocardial perfusion as shown by nuclear scintigraphy even when echocardiography of the arteries was unremarkable. However, a dysfunctional vasculature may not be limited to the heart and CVD may be more widespread than to the coronary arteries, as shown by increased flow-mediated dilatation of the brachial artery after KD.

A study by Kato et al followed 594 patients from the acute KD phase up to 20 years afterwards. In 2.2% of the patient, they found a more widespread disease with extracardiac vascular lesions, although this study was performed at the time that IVIG infusions were not routine.

Carotid intima-media thickness (cIMT) is a well validated, non-invasive surrogate marker for CVD risk in multiple populations.

In several studies cIMT was compared between individuals with a history of KD and unaffected controls, finding an increased cIMT in all KD patients or in CAA-positive patients compared to controls. As compared to controls, CAA-negative subjects showed a positive correlation between cIMT and KD severity of coronary arteritis at the acute stage. Although initially increased, the cIMT in CAA-negative patients normalized at a later age. In contrast, patients with a history of KD complicated by giant CAA showed a trend towards a persistently increased cIMT. These patients may need cardiovascular counseling and follow-up beyond the heart.

Methods
Participants
The study was conducted between October 2001 and December 2014 at the Emma Children's hospital, a tertiary referral center. Subjects with a history of KD, based on...
criteria of the America Heart Association were recruited consecutively during follow-up as outpatients\textsuperscript{8}. Patients in the acute or subacute phase of KD (specified as within six months after the disease) were excluded to minimize the potential confounding influence of the (sub)acute inflammation.

Unaffected siblings of children with KD and other unaffected subjects (family of staff members and staff of our hospital) without a history of KD were eligible as controls, if they did not take any cardiovascular medication. All subjects and/or their parents gave informed consent as approved by the institution’s Research Ethics Board.

**Study protocol**

Blood pressure, body height and weight were measured in participants. Using data of the fifth Dutch growth study performed in 2009 in 20,887 children in The Netherlands, standard deviation scores (SDS) for body mass index (BMI) were calculated based on the age and gender of each participant (http://groeiweb.pgdata.nl/calculator.asp). The medical records of the KD patients were retrospectively reviewed to collect clinical details, i.e.: age at onset of disease, treatment with IVIG, and the presence of CAA. The coronary arteries had been evaluated by two-dimensional echocardiography. CAA were specified by worst-ever z-scores: z-scores adjusted for basal-surface-area\textsuperscript{15,16}. We defined the CAA by their worst-ever score since arteries may be damaged even when the lumen of a previously affected coronary artery has returned to its normal size, indicating a more severe initial systemic vasculitis when compared to children who never had enlargement.

CAA was defined as a coronary z-score \( \geq 2.5 \), a giant aneurysm was defined as a z-score of \( \geq 10 \) or a diameter of \( \geq 8 \) mm.

In the subjects after KD, after an overnight fast, a venous blood sample was taken to measure total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides.

We measured cIMT in the subjects with a history of KD when they visited the outpatient clinic, from the age of five years onwards. Siblings who were at least seven years of age were invited for a cIMT measurement once.

**Carotid IMT measurements**

All subjects were scanned by two experienced and certified sonographers. Over the course of 14 years, 3 ultrasound machines were used: an Acuson 128XP (October 2001-June 2008), an Acuson Aspen (June 2006 - January 2008) and an Acuson Sequioa (January 2008- January 2015). 7 linear array vascular transducers were used on the Acuson 128 XP and Aspen; an 8L5 transducer on the Acuson Sequioa (Siemens AG, Erlangen, Germany).

All scans were performed according to a validated and standardized scanning and image analysis protocol. Briefly, in all subjects the right and left common carotid, carotid bulb and internal carotid arterial wall segments were visualized. Of each segment, a five second, 25 full frames/second cine-loop is temporarily saved on the memory of the ultrasound equipment. As cIMT is known to change slightly (approximately 6-8\%) during the cardiac cycle, cIMT measurements in a fixed point in the arterial cycle is preferred\textsuperscript{21}.

Therefore, all ultrasound instruments were equipped with a dedicated carotid scan protocol where a high persistence scan setting was used. Due to rapid arterial wall movement, this high persistence setting provides a slight movement artifact on the averaged image when the artery is in systole, where at the relative resting phase of the diastole the averaged image of the arterial wall is crisp. From the cine-loop the sonographer was trained and certified to select and save the highest quality and crispest image frame of the segment as a 2x2cm high resolution DICOM still. The scan protocol is described in full elsewhere\textsuperscript{43}.

The image analyses were done off-line. The mean combined cIMT per subject was calculated as follows: \([\text{mean of the left and right common carotid arteries} + \text{[mean of the left and right carotid bulb]} + \text{[mean of the left and right internal carotid arteries]}/3\]. For subjects in whom one of the segments had failed, the cIMT of the same segment of the opposite carotid artery was taken as the mean of both carotid arteries. When both sides had failed, the segment was considered missing.

One image analyst performed all cIMT measurements manually and was blinded for the patient’s case- and CAA-status. Twenty images of the Acuson Sequioa were analyzed twice to assess intra-rater reliability. The intraclass correlation coefficient was 0.92 [95% Confidence Interval (CI); 0.75-0.97] for the mean cIMT.

The Acuson Sequioa, if compared to the less advanced and technically similar XP and Aspen, has improved hardware, software and transducer properties regarding signal to noise ratio, image display/pixel density and image file size and format. To allow for comparison of cIMT data of different machines normalization of measurements is required. We therefore created a correction factor for the scans provided by the different instruments. On the same day, in 10 volunteers the most reliable artery segment, the common carotid artery far walls were scanned on the Acuson Aspen and Sequioa. This comparison revealed systematic difference in cIMT between instruments. Subsequently, we evaluated per comparable age-groups cIMT data of all KD study participants per ultrasound instruments. This statistical evaluation of cIMT subject data revealed the same and systematic differences in cIMT between instruments as those of the volunteer scans. Based on both calculations (delta mean cIMT and measurement differences within the cohort), a correction factor was applied with the most advanced ultrasound instrument, the Acuson Sequioa, as the reference.
Statistics
We evaluated differences in age and gender between patients and controls at the time of the first cIMT measurement by using a Mann-Whitney U and chi-square test, respectively. Differences in the remaining demographics (length, weight, MAP and BMI SDS) between patient with KD and controls were assessed by linear regression analysis, taking family bonds into account by creating a random term. Non-normal variables were log-transformed before analysis. Differences in demographics between KD subgroups were evaluated by ANOVA for parameters with normal distribution, Kruskal Wallis for parameters with a non-normal distribution and chi-square for binary parameters.

A multilevel, repeated measures, linear mixed-effects model was used to evaluate the association between KD and cIMT. In the first model, all KD patients were compared with controls; in the second model, 4 groups were compared: controls, CAA-negative patients, patients with small-medium aneurysms and patients with giant aneurysms. The analyses were adjusted for potentially confounding variables (age, gender, MAP and BMI SDS) which were entered as fixed effects. Also, family relations were taken into account and were adjusted for by creating a random term. As measurements started at age 5 in patients, we calculated the intercept at this age.

To evaluate whether IVIG treatment, total cholesterol, LDL-cholesterol and triglycerides were of significant influence on cIMT, we also performed, in patients only, a linear mixed-effect analysis including these variables.

Multiple imputation was performed for missing blood pressure (30%) and missing segment (4.7%) values. For each missing value, five imputations were performed, based on age, weight, height, BMI SDS and the remaining segments that did not fail. These were subsequently combined into one effect estimate.

A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 22.0 software (IBM Corp). A figure was created using R statistics version 3.0.1 (The R Foundation for Statistical Computing).

Results
A total of 319 subjects with a history of KD were included with a median age of 8.1 years during their first cIMT measurements (range 5.0-43.3 yrs). In these patients, 528 cIMT measurements were performed. Of these cases, 171 KD patients had a single measurement and 148 had two or more cIMT datasets. A total of 150 controls (130 siblings and 20 unrelated individuals) were included with a median age of 12.5 years (range 7.0-31.1 yrs.). The demographic characteristics of patients and controls during the first cIMT measurement are shown in Table 1. The number of cIMT measurements per subgroup are listed in Table 2.

Of the 319 subjects with a history of KD, 241 (75.5%) had a worst-ever coronary artery
The other subjects had CAA, of whom 51 (16.0%) patients had CAA with a z-score of 2.5–10, and 27 (8.5%) patients had giant aneurysms with a z-score of ≥10 and/or a diameter of ≥8 mm. There was no significant difference in gender between patients and controls, but there were significantly more males in the small-medium CAA group (p=0.02) and the giant CAA group (p<0.001) compared to the CAA negative patients.

The clinical characteristics of KD patients and the KD-subgroups are shown in Table 3. There was no significant difference in percentage of IVIG treatment between the groups. Patients with small-medium CAA were significantly younger during their disease compared to patients without CAA. There was no significant difference in age at KD between patients with giant aneurysms and CAA-negative patients.

Overall, patients with a history of KD had a significantly higher estimated marginal mean carotid intima-media thickness (cIMT) compared to controls (0.375 mm [95% CI 0.372–0.378 mm] vs 0.363 mm [95% CI 0.358–0.368 mm]; p<0.001). The model for the longitudinal cIMT data analysis over time shows that patients with a history of KD started with a higher cIMT (Intercept = +0.0145 mm [95% CI: 0.0042–0.0248 mm; p=0.006] at age 5, as compared to controls). There was no difference in increase per age-year (−0.0004 mm [95% CI −0.0014–0.0007 mm; p=0.490] increase per year, compared to controls).

When comparing CAA-negative patients to patients with small-medium CAA and giant CAA, both groups had a comparable intercept at the age of 5, but did have a significantly lower intercept among controls (p<0.01 compared to controls), and then showed a significantly lower increase in cIMT progression per year (p=0.05). There was no significant difference in CAA-negative patients with a score of ≥1.5 or ≥2.5. The other subjects had CAA, of whom 51 (16.0%) patients had CAA with a score of ≥1.5 or ≥2.5, and 27 (8.5%) patients had giant aneurysms with a score of ≥2.5. There was no significant difference in gender between patients with CAA and controls. The clinical characteristics of KD patients and the KD-subgroups are shown in Table 3. Overall, patients with a history of KD had a significantly higher estimated marginal mean cIMT compared to controls (0.375 mm [95% CI 0.372–0.378 mm] vs 0.363 mm [95% CI 0.358–0.368 mm]; p<0.001). The model for the longitudinal cIMT data analysis over time shows that patients with a history of KD started with a higher cIMT (Intercept = +0.0145 mm [95% CI: 0.0042–0.0248 mm; p=0.006] at age 5, as compared to controls). There was no difference in increase per age-year (−0.0004 mm [95% CI −0.0014–0.0007 mm; p=0.490] increase per year, compared to controls).

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we performed a post-hoc analysis to evaluate whether the subjects with giant aneurysms comprised a different group as such, or represented the more extreme phenotype of a spectrum of the disease.

Figure 1 and 2 show the regression lines (95% CI) for cIMT against age, corrected for gender, BMI z-score, MAP and family relations, for controls and patients and for controls and the different patient groups based on CAA worst-ever z-score.

IVIG, total cholesterol, LDL-cholesterol nor triglycerides were significantly associated with cIMT in the multivariable model.

Additional/Post-hoc analyses

Since not all of our patients obtained multiple measurements, we also analyzed the data including only the measurement of patients with follow-up data. This analysis showed a similar result to the analyses including all patients. Patients with small to medium CAA and patients with giant CAA had a comparable intercept at age 5 compared to CAA-negative patients (p=0.131 and p=0.071 respectively), but the progression was significantly increased in patients with giant CAA (0.0035 mm [95% CI 0.0019 to 0.0051 mm]; p<0.001), although the progression was not significantly increased in patients with small-medium CAA (0.0019 mm [-0.0002 to 0.0039 mm]; p=0.81).

After obtaining the results of the increased progression in patients with giant aneurysms but not in patients with small-to-medium sized aneurysms (z-scores 2.5-10),
Our study is the first longitudinal cIMT study in KD and demonstrates that there seems to be a gradual increase in cIMT in KD patients with larger CAA. The cIMT means gradually increased from controls to patients with giant aneurysms. The cIMT of CAA-negative patients showed an increased cIMT following complete convalescence of the acute disease which normalized over time. Patients with small CAA showed an increased (but non-significant) initial cIMT but a comparable cIMT progression parallel to the curves of controls. Patients with medium-sized CAA showed a trend towards an increased initial cIMT with a comparable cIMT progression over time. Evaluating this model, patients with giant CAA and to a much lesser degree patients with medium-sized CAA showed a trend towards a continuously increasing cIMT compared to controls. Patients with giant CAA are most severely affected by the original vasculitis of the coronary vasculature. Our study suggests that these patients should be followed-up for broader cardiovascular assessment beyond the heart. Although these findings need to be confirmed in additional prospective cohort studies, our study also suggests that patients without any enlargement at any point of the disease, may not need lifelong follow-up.

Several factors such as age, gender, BMI, blood pressure, lipids and lifestyle influence cIMT. For the latter, a suitable control group is vital. Therefore we included siblings as controls, having the same environmental and genetic factors. Gender differed between controls and patients, and between the different KD subgroups. There were significantly more males in the small-medium and the giant CAA group compared to the CAA-negative patients. This was expected as male gender is a known risk factor for aneurysms. It should be emphasized that in the model used, gender, BMI, age and blood pressure were adjusted for and lipids were not significantly associated with cIMT in our patient group.

cIMT is strongly correlated to cardiovascular events. A systematic review by Lorenz et al. calculated a relative risk of 1.15 (95% CI 1.12-1.17) per 0.10 mm cIMT difference for myocardial infarction (MI) and a relative risk of 1.18 (95% CI 1.16-1.21) per 0.10 mm cIMT difference for stroke from studies mainly investigating older populations. Eikendal et al. found a hazard ratio of 1.4 per SD increase in cIMT for MI or stroke in adults <45 years of age.

An increased cIMT is seen in children and adolescents with known cardiovascular risk factors such as familial hypercholesterolemia or obesity. An increased cIMT progression was found to be significantly related to the incidence of stroke by Polak et al. In contrast, a meta-analyses of individual patient data from multiple studies have evaluated cIMT after KD in a cross-sectional manner. Some studies found a significantly increased cIMT in KD patients compared to controls, whereas others did not. When comparing CAA-positive patients to controls, some studies found a significantly increased cIMT, and again others did not report any difference. However, most of these studies were small, used variable study designs or lacked a sufficient number of CAA-positive patients and none of the studies had a follow-up of >6 months.

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longitudinal studies in 2012 showed no significant association between cIMT progression and CV events in a mainly middle-aged to older age adults\(^7\). This could be explained by the different methods of measuring cIMT in the different institutes. Another systematic review of randomized controlled trials measuring cIMT change over time, found a statistically significant association between mean change in cIMT over time and the likelihood of developing non-fatal MI (p=0.018) and the combined endpoint of MI and death (p=0.021)\(^8\).

In younger participants, the Bogalusa Heart Study found a significant association between some cIMT segments progression and multiple cardiovascular risk factors such as waist circumference, waist/height ratio, mean arterial pressure, cholesterol and smoking\(^9\). The Young Finns study showed that young adult cIMT progression is associated to risk factors in childhood such as BMI, physical activity and fruit consumption\(^10\). Although the exact risk prediction of (an increased) cIMT in children and young-adults is still unknown, cIMT is clearly correlated to cardiovascular risk factors in a younger population.

Although cIMT is often considered a surrogate marker for (sub)clinical atherosclerosis, it is unlikely that this is also the case in KD patients. First, the cIMT course in CAA-negative patients does not seem to be concordant with atherosclerosis as one would expect the cIMT values to worsen over time instead of normalize. This suggests that the increased cIMT in KD patients originates from a different type of vasculopathy, which is supported by earlier post-mortem (histology) reports that show no accumulation of lipid in the intima or other features consistent with atherosclerosis in coronary arteries\(^11,12\). The etiology and consequences of this KD vasculopathy have yet to be determined. Most cIMT studies in adults relate an increased cIMT to the extent of atherosclerosis. Hence, the cardiovascular risks derived from these studies cannot directly be adopted for the KD population.

Also, Lorenz et al. found a relative risk of MI of 1.15 per 0.10 mm cIMT increase, while our study shows a difference of 0.012 mm between patients and controls\(^8\). Although a lot smaller than in adult atherosclerosis, in patients with giant CAA this difference might increase each year, potentially leading to relevant peripheral vasculature changes over time.

The increase in cIMT at the extreme end of the spectrum is not completely unexpected. Suda et al. described 76 patients with giant aneurysms (>8 mm) in a retrospective cohort with a median follow-up of 19 years, and found that 7 patients had died during follow-up\(^13\). They calculated a 10-, 20- and 30-year survival rate of respectively 95%, 88% and 88%, and a 5-, 15- and 25-year cumulative coronary intervention rate of 28%, 43% and 59%, indicating the coronary arteries are still remodeling years after the acute disease.

Study limitations

Our study has some limitations. First of all, although the cIMT protocol did not change throughout the years, different ultrasound machines were used. We solved this issue by calculating a correction factor between the different machines. Secondly, blood pressure data at the time of cIMT was missing in approximately 30% of children. By imputing the missing data based on many of the known variables, we were able to correct for mean arterial pressure in our model. Since blood pressure did not seem to be of influence on the cIMT because almost all of the children were normotensive, the missing data are unlikely to have influenced the results. Thirdly, patients were stratified based on their “worst-ever” z-score. As the study was conducted in a tertiary referral center, most of the echocardiographies in the acute phase were not performed in our own center, but by pediatric cardiologists in other centers. This may have led to misclassification of some patients in one of the CAA subgroups. Moreover, our study was conducted in a tertiary referral center with inevitable referral bias. In our case, the relatively large number of patient with CAA and especially with giant CAA helped to identify that these patients have the most abnormal response at the peripheral non-cardiac vasculature as well. Finally, our controls and a proportion of our patients did not undergo more than one cIMT measurement. However, by creating a multilevel, repeated-measures, linear-mixed-effects model we could use all cIMT measurements to create a large study group.

Even though this is the largest study on cIMT in patients after KD, there is a possible lack of power in the subgroups. The arterial walls of the young encompass approximately 0.4 mm, where cIMT as measured by B-mode ultrasound has an axial resolution of around 0.04-0.05 mm, hence to detect submillimeter differences, large groups sizes are required. We observed a trend towards significance in both intercept and progression in different subgroups, however, as significance is dependent on the sample size and because in both groups the mean was indeed significantly increased, it is likely that a significant finding will be present in larger groups. Therefore, our data need confirmation in (a very) large number of participants with longitudinal follow-up, in particular in the smaller subgroups of CAA-positive patients.

Conclusion

The cIMT of KD patients is increased compared to healthy controls. Although the cIMT of CAA-negative children is initially increased, the values have normalized at a later age, suggesting vascular repair of a generalized vasculopathy distinctive from atherosclerosis. Patients with a history of KD complicated by giant and to a lesser degree by medium-sized CAA have a trend towards a continued increased cIMT, suggesting a more severe impact on the arterial wall. Until more data become available, these patients need cardiovascular counseling and follow-up beyond the heart.
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References


Cardiovascular imaging techniques in children and adults following Kawasaki disease


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Kawasaki disease (KD) is a paediatric vasculitis with coronary artery aneurysms (CAA) as its main complication. Two guidelines exist regarding the follow-up of patients after KD, by the American Heart Association and the Japanese Circulation Society. After the acute phase, CAA-negative patients are checked for cardiovascular risk assessment or with ECG and echocardiography until 5 years after the disease. In CAA-positive patients, monitoring includes myocardial perfusion imaging, conventional angiography and CT-angiography. However, the invasive nature and high radiation exposure do not reflect technical advances in cardiovascular imaging. Newer techniques, such as cardiac MRI, are mentioned but not directly implemented in the follow-up.

Cardiac MRI can be performed to identify CAA, but also evaluate functional abnormalities, ischemia and previous myocardial infarction including adenosine stress-testing. Low-dose CT angiography can be implemented at a young age when MRI without anaesthesia is not feasible. CT calcium scoring with a very low radiation dose can be useful in risk stratification years after the disease.

By incorporating newer imaging techniques, detection of CAA will be improved while reducing radiation burden and potential complications of invasive imaging modalities. Based on the current knowledge, a possible pathway to follow-up patients after KD is introduced.

Introduction
Kawasaki disease (KD) is an acute paediatric vasculitis that mainly affects children younger than 5 years of age. Although the exact cause is still unknown, it is thought to be caused by an infectious agent in genetically predisposed children. This fits with the observed epidemiology of seasonal occurrence throughout the northern hemisphere, the increased incidence in children of Japanese descent and the established association with specific genetic polymorphisms. In about 25% of untreated patients, coronary artery aneurysms (CAA) will develop during the acute phase of KD. Since the introduction of high-dose intravenous immunoglobulin as effective treatment, the percentage of CAA has dropped to 5-7% for patients treated within 10 days after fever onset. A proportion of the patients is not treated within 10 days and for them the rate of developing aneurysms may be higher.

During CAA formation it is thought that an influx of inflammatory cells leads to dissociation and disruption of the medial and internal elastic lamina layer. Even when the lumen of the artery returns to its normal size, the artery wall remains damaged, although the extent of the damage varies among patients. In one autopsy study, active remodelling of the artery was seen many years after the acute disease in children with aneurysms. The KD-related vasculopathy during follow-up with characteristic myointimal proliferation and/or layering of thrombus can result in progressive stenosis, which may lead to ischemic cardiomyopathy.

Calcification of the damaged artery is progressive after the acute phase of the disease and may develop in coronary arteries with lesions that persist or, although rarely, in transiently dilated arteries when the vessel lumen has normalized. The extensive calcification of the coronary artery wall is typical of the pathology observed in the remodelled lesional wall (Figure 1).

CAA are diagnosed by echocardiography during the acute or subacute phase of the disease. CAA are traditionally classified according to the definitions of the Japanese Ministry of Health: a luminal diameter of ≥ 3 in children <5 years of age, >4 mm in children ≥ 5 years of age, or a diameter 1.5 times the size of an adjacent segment or an irregular lumen in children ≥ 5 years of age. A giant aneurysm is defined as an aneurysm with an internal diameter of ≥ 8 mm or a diameter >4 times that of an adjacent segment in children ≥ 5 years old. However, over the past years it has become clear that z-scores, adjusted for body-surface area, may be a better indicator of any serious enlargement. Using this approach, a z-score of ≥ 2.5 is considered an aneurysm; a z-score of ≥ 10 a giant aneurysm.

It is clear that a 6 mm aneurysm in a 6-month old child represents a more severely damaged artery than a 6 mm aneurysm in a 6-year old child. As z-scores might differ from the traditional definitions, especially in younger and smaller children, this may alter their (long-term) therapy and monitoring.
PART I

In both guidelines, patients are risk-stratified based on the severity of the coronary artery lesions and likelihood of complications, including myocardial ischemia and congestive heart failure.

In children without CAA or with early, transient coronary artery dilatations, the AHA advises echocardiograms at initial diagnosis and 2 and 6-8 weeks later. A repeat echocardiogram at 1 year is considered optional. Subsequently, only periodic assessment and counselling about cardiovascular risk factors are recommended. To evaluate patients without CAA, the JCS advises an ECG and echocardiogram at 1, 2, 6 and 12 months, and a final evaluation at 5 years with an exercise ECG.

For patients diagnosed with persisting CAA after the acute phase, both guidelines advise a more intensive and prolonged follow-up with additional imaging modalities. In children with small to medium aneurysms, the AHA advises an annual routine follow-up and biennial stress testing with myocardial nuclear perfusion scans. In case of large and/or multiple or complex aneurysms within one artery, the AHA recommends an invasive coronary angiography (CAG) to be performed routinely 6-12 months after the disease onset and repeated if the non-invasive stress test or clinical signs suggest myocardial ischemia. It should be noted that these guidelines were last revised in 2004 and many aspects of clinical care and imaging have changed during this interval.

The JCS has recently published revised guideline in which the committee recommends follow-up for patients with small aneurysms at 30 days after the acute illness with an echocardiogram and ECG every 3 months until the dilatation has disappeared. Patients with medium-sized aneurysms should be more intensely evaluated, i.e. every 1-3 months with (exercise) ECG, echocardiography and chest X-ray until dilatation is no longer observed and should undergo MDCT or MR Coronary Angiography (MRCA) every five years. This scheme differs from the AHA guidelines.

For those with larger CAA, JCS advises a follow-up in a risk-stratified manner using methods that are largely overlapping the AHA recommendations. There are no specific recommendations for imaging modalities.

Concerns

There are two main drawbacks to the current guidelines for follow-up imaging of patients with KD. These guidelines are summarized in Table 1 and 2, starting at one year after the acute disease. In both guidelines, patients are risk-stratified based on the severity of the coronary artery lesions and likelihood of complications, including myocardial ischemia and congestive heart failure.

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1. The invasive nature and high radiation exposure of the advised follow-up modalities do not reflect technical advances in the field of cardiovascular imaging and result in possible complications and unnecessary hazardous radiation exposure.
2. Coronary aneurysms, especially those more distal in the coronary tree, can be missed by echocardiography, potentially leading to underestimation of disease severity.

Current guidelines

Among the many issues concerning clinical management of patients with a history of KD, several controversies exist. One of these is the timing and need for long-term follow-up. Two consensus guidelines have been published, one by the American Heart Association (AHA) in 2004 and one by the Japanese Circulation Society (JCS) in 2014. These guidelines are summarized in Table 1 and 2, starting at one year after the acute disease. In both guidelines, patients are risk-stratified based on the severity of the coronary artery lesions and likelihood of complications, including myocardial ischemia and congestive heart failure.

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**Table 1** Summary of AHA guidelines regarding the follow-up of patients with Kawasaki disease, starting at 1 year

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Diagnostic testing</th>
<th>Interval</th>
<th>Invasive testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CAA</td>
<td>Cardiovascular risk assessment</td>
<td>5 years</td>
<td>None</td>
</tr>
<tr>
<td>Transient CAA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cardiovascular risk assessment</td>
<td>3-5 years</td>
<td>None</td>
</tr>
<tr>
<td>Small-Medium CAA (&lt;3 mm but &lt;6 mm, z-score 3-7)</td>
<td>Cardiovascular risk assessment, Echocardiogram + ECG, Stress test with MPI</td>
<td>1 year</td>
<td>Invasive CAG if non-invasive test suggests ischemia</td>
</tr>
<tr>
<td>Large CAA (≥6 mm) or Multiple or complex CAA in 1 artery</td>
<td>Echocardiogram + ECG, Stress test with MPI</td>
<td>½ year</td>
<td>Invasive CAG after 6-12 months and if any test or clinical finding suggests ischemia</td>
</tr>
<tr>
<td>Coronary artery obstruction</td>
<td>Echocardiogram + ECG, Stress test with MPI</td>
<td>½ year</td>
<td>Invasive CAG for therapeutic options and if new onset or worsening myocardial ischemia is suggested</td>
</tr>
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</table>


<sup>a</sup> Disappearing within 6-8 weeks after the onset of Kawasaki disease. CAG: conventional angiography; MPI: myocardial perfusion imaging.

**Table 2** Summary of JCS guidelines regarding the follow-up of patients with Kawasaki disease, starting at 1 year

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Diagnostic testing</th>
<th>Interval</th>
<th>Invasive testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>No or transient CAA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Exercise ECG + echocardiogram</td>
<td>Once, 5 years after disease&lt;sup&gt;b&lt;/sup&gt;</td>
<td>None</td>
</tr>
<tr>
<td>Small CAA (≤4 mm) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>Exercise ECG + echocardiogram</td>
<td>Annual until age 7 3 months (until normalisation)</td>
<td>None</td>
</tr>
<tr>
<td>- Regressed</td>
<td>(Exercise) ECG + echocardiogram</td>
<td>Annual until age 7 3 months (until normalisation)</td>
<td>None</td>
</tr>
<tr>
<td>- Persisting</td>
<td>(Exercise) ECG + echocardiogram</td>
<td>Annual until age 7 3 months (until normalisation)</td>
<td>None</td>
</tr>
<tr>
<td>- Regressed or persisting</td>
<td>In patients ≥10 years after onset, consider MDCT or MRCA at final evaluation.</td>
<td>Annual until age 7 3 months (until normalisation)</td>
<td>None</td>
</tr>
<tr>
<td>Medium CAA (&gt;4-8 mm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ECG + echocardiogram, X-ray + exercise ECG when necessary/feasible, MDCT or MRCA, ECG + echocardiogram</td>
<td>5 years</td>
<td>Selective CAG on individual basis</td>
</tr>
<tr>
<td>A: CAA &gt;4-6 mm</td>
<td>ECG + echocardiogram, X-ray + exercise ECG when necessary/feasible, MDCT or MRCA, ECG + echocardiogram</td>
<td>3-6 months</td>
<td>Selective CAG on individual basis</td>
</tr>
<tr>
<td>- Regressed</td>
<td></td>
<td>Annual</td>
<td>Selective CAG on individual basis</td>
</tr>
<tr>
<td>- Persisting</td>
<td></td>
<td>5 years</td>
<td>Selective CAG on individual basis</td>
</tr>
</tbody>
</table>
For this reason the use of alternative imaging methods should be considered and we suggest a feasible, accurate and sustainable follow-up scheme for KD as outlined below.

**Table 2 Continued**

<table>
<thead>
<tr>
<th>CAA 6 - &lt; 8 mm</th>
<th>Diagnostic testing</th>
<th>Interval</th>
<th>Invasive testing</th>
</tr>
</thead>
</table>
| - Regressed    | ECG + echocardiogram  
X-ray + exercise ECG when necessary/feasible  
MDCT or MRCA  
Appropriate combination of techniques | Annual | Invasive CAG once during convalescence and time of disappearance of dilatation |
| - Persisting   | ECG + echocardiogram  
X-ray + exercise ECG when necessary/feasible  
MDCT or MRCA  
Appropriate combination of techniques | 3-6 months | Invasive CAG once during convalescence and at time of disappearance of dilatation |
| Giant CAA (≥ 8 mm) | Tailor-made treatment with appropriate combination of (exercise) ECG, echocardiogram and other techniques | 3-6 months | Invasive CAG during early convalescence phase |

MDCT: Multidetector CT, MRCA: MR Coronary Angiography.  
a. Measured at 30 days after the onset of KD.  
b. Additional follow-up can be scheduled individually through consultation between patient and physician.  
c. Imaging techniques include stress echo and exercise testing, stress myocardial perfusion scintigraphy, invasive coronary angiography (CAG), Intravenous Ultrasound, Cardiac Magnetic Resonance Imaging, Magnetic Resonance Angiography and Multidetector CT.
abnormalities and myocardial scar. Standard, commercially available, CMRI techniques are used with fast single shot T1-weighted gradient echo sequences (e.g. Turbo-FLASH, TFE) for perfusion imaging and segmented inversion recovery sequences for myocardial scar imaging18-19.

We introduced this 2-step protocol because perfusion abnormalities and ischemia upon adenosine stress were detected only in patients with persistent CAA.

Based on current data, it is unlikely that patients without CAA will ever develop clinically significant cardiovascular changes from their KD. However, in patients with in children with missed CAA by echocardiography, long-term complications can occur.

**Low-radiation dose CT angiography**

Although CMRI is a feasible technique in early adolescence, assessment of younger children with CAA often requires general anaesthesia and is therefore less suitable.

ECG-gated multislice CT is an attractive alternative imaging technique for the younger patient. In teenagers with a history of KD, CT-angiography confirmed all aneurysms, stenoses and occlusions previously demonstrated by invasive CAG20. In another study, aneurysms were detected by CT-angiography in young children with a history of KD, that had been missed by echocardiography21.

Although effective, traditional CT-angiography carries a high radiation exposure, especially harmful for young children. Low-radiation dose CT-angiography with prospective ECG triggering is increasingly available. The use of next generation scanners decreases the radiation burden from a median of 6.9 mSv per scan down to a median of 1 mSv on a 128 dual-source CT scanner22. New imaging protocols can be used to reduce radiation dose of which the use of prospective instead of retrospective ECG-triggering is most important. The use of a single heart beat (high-pitch) protocol has been shown to result in a reduction of the radiation dose requirements even further. On the other hand, studies are conflicting as to whether the image quality is reduced when using prospective ECG triggering is most important. The use of a single heart beat (high-pitch) protocol has been shown to result in a reduction of the radiation dose requirements even further.

Although effective, traditional CT-angiography carries a high radiation exposure, especially harmful for young children. Low-radiation dose CT-angiography with prospective ECG triggering is increasingly available. The use of next generation scanners decreases the radiation burden from a median of 6.9 mSv per scan down to a median of 1 mSv on a 128 dual-source CT scanner22. New imaging protocols can be used to reduce radiation dose of which the use of prospective instead of retrospective ECG-triggering is most important. The use of a single heart beat (high-pitch) protocol has been shown to result in a reduction of the radiation dose requirements even further. On the other hand, studies are conflicting as to whether the image quality is reduced when using prospective ECG triggering is most important. The use of a single heart beat (high-pitch) protocol has been shown to result in a reduction of the radiation dose requirements even further.

By not performing frequent echocardiography after the acute phase, costs are kept to a minimum. Furthermore, by detecting missed aneurysms or other lesions on CMRI, possible complications such as myocardial infarction can be prevented. It must be stated that this pathway will depend on the available technical quality and experience of a centre with CMRI which varies across centres.

For patients with CAA, the pathway must be customized depending on the extent of the lesions. For instance, a young child with giant aneurysms and suspected ischemia will require invasive or CT-angiography, as well as MRI under general anaesthesia, repeated regularly as an individual approach, to obtain the best interpretation of CAA and functional stenosis.
CHAPTER 6 CARDIOVASCULAR IMAGING TECHNIQUES IN CHILDREN AND ADULTS FOLLOWING KAWASAKI DISEASE

PART I

a. Echocardiogram of a giant aneurysm of the left main coronary artery (LMCA) and LAD.
b. Stress and rest SPECT Technetium-99M scan (myocardial perfusion scan) demonstrates ischemia of the inferior and septal wall.
c. Conventional CAG shows a giant aneurysm of the LAD and a smaller aneurysm of the right circumflex artery (RCX).
d. Cardiac MRI shows an aneurysm of the LAD.
e. Cardiac MRI indicates a myocardial infarction of the infero-posterior wall.
f. Multi-slice CT contrast-enhanced angiography with calcified aneurysms of the proximal LAD and right coronary artery (RCA).
g. CT calcium-score with calcifications of the proximal LAD.
h. 3D-CT angiography with a calcified aneurysm of the RCA.

FIGURE 2 Imaging techniques for the follow-up of Kawasaki disease

a. When information is lacking about coronary arterial aneurysms (CAA) status, calcium score may be indicated as a screening method. If positive, a CMRI with adenosine should be performed.
b. Long-term follow-up (cardiovascular counselling) of risk group 1 may be dictated by national health care policies and future studies.
c. According to the availability and experience of a centre with low-dose CT angiography.
d. Which of the different revascularization options improves prognosis best is unclear to date.
e. Additional tests to evaluate for progression to stenotic lesions.

FIGURE 3 Flowchart for the monitoring of Kawasaki disease with current imaging modalities starting at 1 year after the disease

a. Flowchart for the monitoring of Kawasaki disease with current imaging modalities starting at 1 year after the disease.
In summary, we believe that the current guidelines for follow-up of patients with KD need to be revised. By incorporating new imaging techniques in our guidelines, detection of CAA will be improved, while reducing the radiation burden and potential complications of invasive angiography. This may enable better adherence to the guidelines in clinical practice and improve follow-up of patients into adulthood.

Acknowledgements
We are grateful to Dr. Hein Verberne (Academic Medical Centre, Amsterdam, The Netherlands) and Chisato Shimizu (University of California, San Diego, USA) for their help with the selection of images.

In contrast to infants and children, adolescents and adults are able to report cardiac symptoms of ischemia. Asymptomatic adults with aneurysms must be followed for early signs of ischemia upon (stress) echocardiography, but the frequency of additional imaging studies can largely be guided by symptoms. Since echocardiography in general is insensitive for evaluating stenosis, symptomatic adults require other imaging techniques.

In addition to the techniques mentioned above, in a selected group of patients conventional CAG can be considered when a catheter-based intervention is contemplated. Invasive CAG can be combined with fractional flow reserve to obtain hemodynamic and structural data to help guide therapeutic decisions. These tests for interventional decision-making have not often been reported in KD and are beyond the scope of the article.

Future studies
Future studies are needed to evaluate the risks and benefits of different cardiovascular imaging modalities and the most appropriate timing of such imaging to optimize care for KD patients. Evidence is lacking about the long-term effects of KD, especially in children without prior evidence of CAA. We might not appreciate late cardiovascular sequelae that may become clinically important later in life. In order to answer these questions, long-term follow-up is required.

<table>
<thead>
<tr>
<th>Imaging technique</th>
<th>Advantage(s)</th>
<th>Disadvantage(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echocardiography</td>
<td>Non-invasive</td>
<td>Distal coronary arteries not visible</td>
</tr>
<tr>
<td></td>
<td>Cheap</td>
<td>Invasive, possible complications</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Need of anaesthesia</td>
</tr>
<tr>
<td>CAG</td>
<td>Complete image of coronary tree</td>
<td></td>
</tr>
<tr>
<td>CMRI</td>
<td>Visualisation of distal aneurysms</td>
<td>Need of anaesthesia in younger children</td>
</tr>
<tr>
<td></td>
<td>Functional assessment</td>
<td></td>
</tr>
<tr>
<td>CT-angiography</td>
<td>Visualisation of distal aneurysms and stenosis</td>
<td>Widely available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiation exposure</td>
</tr>
<tr>
<td>CT-calciumscore</td>
<td>Visualisation of calcifications late after disease</td>
<td>Low-radiation exposure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not suitable for aneurysm information</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Only applicable late after acute disease</td>
</tr>
</tbody>
</table>

CAG: conventional angiography; CMRI: Cardiac MRI
References

PART II
GIANT ANEURYSMS
Giant aneurysms: a gender-specific complication of Kawasaki disease?


Journal of Cardiology, 2017, in press
**Background:** Kawasaki disease (KD) is a pediatric vasculitis of unknown origin. Its main complication is the development of coronary artery aneurysms (CAA) with giant CAA at the end of the spectrum.

**Methods:** In this cohort study, we evaluated the association between patient characteristics and the development of giant CAA based on z-scores. Multivariable, multinomial logistic regression analysis was used to identify variables associated with giant CAA.

**Results:** A total of 301 KD patients, comprising of 216 patients without enlargement, 45 with small-sized, 19 with medium-sized, and 21 with giant CAA with all echocardiographies at our center were retrospectively included. Remarkably, 95% of patients with giant CAA were boys. In addition to ‘no/late intravenous immunoglobulin (IVIG) treatment’, ‘male gender’ (OR 16.23, 95% CI 1.88-140.13), ‘age <1 year’ (OR 7.49, 95% CI 2.29-24.46), and ‘IVIG re-treatment (9.79, 95% CI 2.79-34.37)’ were significantly associated with an increased risk of giant CAA, with patients without enlargement as reference. Compared to patients medium-sized CAA, ‘IVIG re-treatment’ was significantly associated with giant CAA. The majority of giant CAA continued to increase in size during the first 40 days.

**Conclusions:** We identified risk factors associated with an increased risk of giant CAA. The difference in variables between the giant CAA group and the other CAA subgroups suggests a separation between patients with the treatment-resistant giant CAA and the other IVIG-responsive patients, in which gender may be factored as a most relevant genetic trait. The increase in size during the first 2 months indicates the need for repeated echocardiography.

**Introduction**

Kawasaki disease (KD) is a pediatric vasculitis mainly occurring in children under the age of 5. Even though the disease was first described in 1967, its origin is still unknown. The main complication of KD is the development of coronary artery aneurysms (CAA). Treatment with intravenous immunoglobulins (IVIG) has been found to significantly reduce the risk of CAA formation.

At the extreme end of the spectrum of CAA are giant CAA, which severely affect the prognosis of the patient due to a higher risk of myocardial ischemia, infarction and death. (Giant) CAA can be defined by Japanese criteria, which are based on the absolute diameter of the coronary artery. For example, following this criterion, giant CAA are defined as a lumen of ≥8 mm. In the past years it has become clear that z-scores, diameters adjusted for basal body-surface area (BSA), may be a better measure for abnormality. By using these z-scores, small dilatations and/or giant CAA can be classified as such in young children without being above the absolute diameter threshold.

Multiple studies have looked at risk factors for the development of giant CAA, yet all of these studies are performed in a Japanese population with the use of Japanese criteria for CAA, thus possibly excluding small children with giant CAA according to z-scores from the giant CAA group. Also, studies have shown that Japanese risk factor scores, often used to determine treatment, have a low sensitivity to predict IVIG resistance in American and Western Mediterranean populations and differed substantially between ethnicities. As a consequence, these risk factor scores will not apply to children with a Western background.

The aim of this study is thus to evaluate possible risk factors for giant CAA based on z-scores in a Western population of KD children as well as the location and rate of development following the onset of fever.

**Materials and methods**

**Study population and data collection**

The Academic Medical Center in Amsterdam (The Netherlands) is a tertiary referral center for patients with KD, and patients visit our multidisciplinary outpatient clinic for cardiology and/or for (immunologic) long-term follow-up. It is also the primary center for echocardiography during the acute phase for children being admitted to hospitals in the proximity of our center. These hospitals do not have pediatric cardiologists available, and therefore refer children to our center for cardiac evaluation.

Patients with KD who visited our (multidisciplinary) outpatient clinic between January 1999 and December 2015 were eligible. Patients were included if their echocardiography monitoring during the acute phase and follow-up had been performed at our center.

We extracted retrospectively the clinical details from the medical records, i.e.: gender,
age at disease onset, complete or incomplete disease presentation, treatment with IVIG including the day of first IVIG treatment, IVIG re-treatment, treatment with steroids and presence of CAA. In our hospital, standard KD-therapy consists of a single IVIG dose of 2 g/kg, given over 8-12 hours. The criteria for IVIG-retreatment are persisting or recurrent fever, 36-48 hours after the original IVIG-treatment.

Coronary artery aneurysms
At our hospital, coronary arteries are routinely evaluated by two-dimensional echocardiography in the acute phase of KD, i.e. in the first week and second week. If no abnormalities were detected, another echocardiography is made in week 6, after 6-12 months and every 3-5 years during follow-up. In case of an abnormality, echocardiography is repeated every 1 or 2 weeks, depending on the extent of the abnormality. All echocardiographies are performed by experienced pediatric cardiologists. From echocardiography reports, we registered information on CAA status. We specified CAA by the worst z-score within the first 6 weeks. BSA and z-scores were calculated according to the ‘Boston method’4,5. The z-scores of the left main coronary artery (LMCA), right coronary artery (RCA) or left anterior descending artery (LAD) were calculated. Z-scores define small CAA as a z-score of 2.5 to 5, medium CAA as a z-score of 5 to 10, and giant CAA as a z-score of ≥10 or a diameter of ≥8 mm. If no exact diameters were available, we categorized these patients based on the description of the echocardiography report.

After the CAA status of all patients was established, we aimed to visualize the development of giant CAA, calculating the z-scores of the coronary arteries until the maximal z-score of the artery was reached during follow-up echocardiography during the (sub)acute stage of the disease. The z-scores were depicted against time, to evaluate how the size of the CAA evolved over time. Also, we aimed to determine on which day the CAA approximately crossed the line of a z-score of 10 and thus ‘became giant’. Therefore, we assumed the z-score was zero before disease onset. By drawing a line from zero to the z-score at the first echocardiography and by connecting the z-scores from the different echocardiographies we estimated the days the CAA acquired the dimensions of a giant CAA (i.e. a z-score of ≥10).

Statistics
We compared demographic and clinical characteristics of patients with giant CAA with patients without enlargement, small CAA or medium CAA by univariable multinomial logistic regression analyses. Variables included were complete or incomplete disease presentation, age, gender, IVIG re-treatment, and day of first IVIG treatment. IVIG treatment and timeliness of IVIG-treatment were included because these variables are known to decrease the chances of developing CAA(1). Other variables were chosen based on existing literature and clinical relevance4-7.

Complete disease presentation was defined as the presence of 5 or more KD criteria. Age during day of onset was divided in 3 groups, i.e. <1 year, 1-5 year and >5 years. Day of first IVIG treatment was also subdivided in 4 categories; i.e. 1-5 days, 6-10 days, 11-15 days and more than 15 days after disease onset or no IVIG treatment. Although it is possible that a late gift of IVIG will improve the course of already existing (giant) CAA, it is unlikely that it will prevent the development of such abnormalities all-together. Hence we believe that it will not influence the outcome of this study. Therefore, we categorized all patients who received IVIG after day 15 in the same category as patients who did not receive IVIG at all.

Variables with a p-value of 0.2 or less from a univariable analysis were entered into a multivariable multinomial logistic regression model. Results are presented as odds ratios (OR) and their 95% confidence intervals (CI).

Statistical analyses were performed using SPSS version 22.0 software (SPSS Inc Chicago, IL).

Results
Study population
In total, 676 KD patients were followed-up at our center. We included those patients who obtained their echocardiographies in the acute phase at our center and these 301 KD patients comprised our study population. The remaining 375 patients obtained echocardiographies at other centers during the acute phase. The median (interquartile range, IQR) age of disease onset was 2.1 years (0.9-4.0 years) and 184 (61.1%) of patients were boys. Seventy-four percent of patients were of Caucasian or mixed Caucasian (with one parent of another ethnicity) descent. The remaining patients were of Mediterranean (Turkish or Moroccan), Surinamese (Indo-Surinamese or African-American) or Asian descent. Of all children, 282 (93.7%) were treated with IVIG and out of these 282, 82% were treated within 10 days after disease onset. 20% received a second IVIG treatment and 7% received additional steroid treatment. Almost all children were treated with aspirin.

We were able to calculate z-scores in 271 patients. Of the remaining 30 patients the exact diameters were not recorded, but 29 were described as being normal and only 1 had been described as having a small dilatation. When adding these patients to the ‘no enlargement’ and the ‘small-sized CAA group’, respectively, 216 (71.8%) patients had no enlargement, 45 (15.0%) had small CAA, 19 (6.3%) had medium CAA and 21 (7.0%) had giant CAA. In Figure 1, boxplots of the median worst z-score with IQR of the 4 CAA subgroups are shown. Demographic and clinical characteristics according to the different subgroups are shown in Table 1.
Association between patient characteristics and giant CAA

We evaluated the association between patient characteristics and giant CAA. In Table 2, the ORs and their 95% CIs are shown for the different patient characteristics in patients with giant CAA compared with patients without enlargement, with small-sized CAA and with medium-sized CAA. In multivariable analyses, ‘male gender’, ‘young age (<1 year) during the acute disease’, ‘IVIG re-treatment’, and ‘IVIG treatment after day 10 (late treatment)’ were significantly associated with an increased risk of developing a giant CAA, when compared to patients without enlargement. Compared with patients with small CAA, a young age, IVIG re-treatment and treatment with IVIG after day 15 or no IVIG were significantly associated with an increased risk of developing giant CAA. Compared to patients with medium CAA, a second IVIG treatment was significantly associated with the development of giant CAA.

Since many patients did not receive IVIG within the recommended 10 days, we further explored the association between patient characteristics and giant CAA in patients who

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**TABLE 1**

Demographic and clinical characteristics of the KD patients per CAA group based on z-scores

<table>
<thead>
<tr>
<th>CAA group</th>
<th>n=216</th>
<th>n=45</th>
<th>n=19</th>
<th>n=21</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Giant CAA (z-score ≥10)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male gender, no (%)</td>
<td>120 (55.6)</td>
<td>31 (69)</td>
<td>13 (68)</td>
<td>20 (95)</td>
</tr>
<tr>
<td>Age (yrs)*</td>
<td>2.4 (1.1-4.0)</td>
<td>1.9 (0.6-3.9)</td>
<td>1.6 (0.4-4.6)</td>
<td>0.8 (0.3-1.6)</td>
</tr>
<tr>
<td>Complete KD, no (%)</td>
<td>177 (81.9)</td>
<td>32 (73)</td>
<td>13 (68)</td>
<td>14 (67)</td>
</tr>
<tr>
<td>IVIG treatment, no (% treated)</td>
<td>199 (92)</td>
<td>42 (93)</td>
<td>19 (100)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>Day of IVIG treatment†</td>
<td>7 (6-9)</td>
<td>6 (6-9)</td>
<td>8 (6-19)</td>
<td>11 (6-28)</td>
</tr>
<tr>
<td>IVIG re-treatment, no (%)</td>
<td>34 (15.8)</td>
<td>9 (22)</td>
<td>5 (26)</td>
<td>11 (52)</td>
</tr>
<tr>
<td>Steroid treatment, no (%)</td>
<td>5 (2.3)</td>
<td>5 (11)</td>
<td>3 (16)</td>
<td>8 (38)</td>
</tr>
<tr>
<td>Maximal z-score</td>
<td>1.1 (0.4-1.8)</td>
<td>3.0 (2.7-3.7)</td>
<td>7.0 (5.3-7.7)</td>
<td>17.0 (14.3-21.4)</td>
</tr>
<tr>
<td>Maximal diameter</td>
<td>2.4 (2.0-2.7)</td>
<td>3.3 (2.7-3.5)</td>
<td>4.0 (3.6-5.4)</td>
<td>6.5 (5.7-7.5)</td>
</tr>
</tbody>
</table>

* Median (Interquartile range) † Counted from the first day of fever

CAA=Coronary artery aneurysm, IVIG=Intravenous immunoglobulins, KD=Kawasaki disease.
<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of KD (reference: complete)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete</td>
<td>1.00 (0.98-1.03)</td>
<td>1.00 (0.96-1.04)</td>
</tr>
<tr>
<td>Incomplete</td>
<td>2.60 (0.95-7.02)</td>
<td>0.06 (0.36-1.00)</td>
</tr>
<tr>
<td>Gender (reference: female)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.07 (0.01-0.70)</td>
<td>0.49 (0.14-1.69)</td>
</tr>
<tr>
<td>Female</td>
<td>1.00 (0.98-1.03)</td>
<td>1.00 (0.96-1.04)</td>
</tr>
<tr>
<td>IVIG re-treatment (reference: no re-treatment)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.00 (0.98-1.02)</td>
<td>0.57 (0.19-1.67)</td>
</tr>
<tr>
<td>No</td>
<td>1.00 (0.98-1.03)</td>
<td>1.00 (0.96-1.04)</td>
</tr>
<tr>
<td>Age (reference: age 1-5 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt;1 year</td>
<td>0.00 (0.00-0.01)</td>
<td>0.00 (0.00-0.01)</td>
</tr>
<tr>
<td>Age &gt;5 years</td>
<td>0.00 (0.00-0.01)</td>
<td>0.00 (0.00-0.01)</td>
</tr>
<tr>
<td>Day of IVIG (reference: Day 6-10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3-5</td>
<td>1.00 (0.98-1.02)</td>
<td>0.61 (0.36-1.04)</td>
</tr>
<tr>
<td>Day 11-15</td>
<td>0.00 (0.00-0.01)</td>
<td>0.00 (0.00-0.01)</td>
</tr>
<tr>
<td>Day &gt;15 or no IVIG</td>
<td>1.00 (0.98-1.02)</td>
<td>0.61 (0.36-1.04)</td>
</tr>
</tbody>
</table>

CI = Confidence Interval, IVIG = Intravenous Immunoglobulins, KD = Kawasaki Disease, OR = Odds Ratio.
We found that ‘male gender’, ‘IVIG re-treatment’, ‘age <1 year’ in addition to ‘late or no IVIG treatment’ were significantly associated with an increased risk of developing giant CAA when compared to patients without enlargement. Furthermore, we demonstrated that giant CAA most frequently affect the RCA and that the majority of giant lesions continued to increase in the first 20-40 days after disease onset.

Multiple studies in Japan have studied risk factors for giant CAA. Nakamura et al and Sudo et al published 3 studies examining data from the 15th (1997-1998) plus 16th (1999-2000) and the 19th (2005-2006) nationwide surveys in Japan including children treated with IVIG within 10 days; and giant CAA were defined as a diameter of ≥8 mm6,7,13. Although the data on gender remained inconclusive, these studies found ‘young age’ (i.e. <1 year) and a ‘second IVIG dose’ to be significantly associated with an increased risk of developing giant CAA.

We should notice that direct comparison of these Japanese studies with Western studies is difficult because of the possible differences in clinical practice and data analysis. The current treatment regimen for KD in Japan consists of a single, high dose infusion of IVIG3,14. Conversely, at the era of the before mentioned studies, not all children received IVIG or used the same IVIG dosing regimen due to the medical insurance system in Japan6. This is in part caused by the use of scoring systems for IVIG resistance, which are not applicable to patients with a Western background8,10. Finally, these studies defined giant CAA as an artery diameter of ≥8 mm. We used z-scores adjusted for BSA instead, which may be a better indicator of abnormality4,5. Also, in previous studies patients with

### TABLE 3

Diameters and z-scores of giant CAA per coronary artery

<table>
<thead>
<tr>
<th>Coronary Artery</th>
<th>Diameters (mm) of giant CAA</th>
<th>Z-scores of giant CAA</th>
<th>No of patients with giant CAA*</th>
<th>No of patients with medium CAA*</th>
<th>No of patients with small CAA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA</td>
<td>6.7 (5.5-8.0)</td>
<td>10.2 (13.2-18.3)</td>
<td>17</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>LMCA</td>
<td>6.5 (5.8-7.5)</td>
<td>12.5 (10.5-15.0)</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>LAD</td>
<td>5.7 (4.7-7.2)</td>
<td>10.6 (14.3-23.7)</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCA/LMCA/LAD</td>
<td></td>
<td></td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RCA/LAD</td>
<td></td>
<td></td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>RCA/LMCA</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Four patients had a single giant CAA of the RCA, three of the LAD and one of the LMCA; six patients had a single medium CAA of the LMCA and two patients had a single small CAA of the LMCA

**Discussion**

We found that ‘male gender’, ‘IVIG re-treatment’, ‘age <1 year’ in addition to ‘late or no IVIG treatment’ were significantly associated with an increased risk of developing giant CAA when compared to patients without enlargement. Furthermore, we demonstrated that giant CAA most frequently affect the RCA and that the majority of giant lesions continued to increase in the first 20-40 days after disease onset. Multiple studies in Japan have studied risk factors for giant CAA. Nakamura et al and Sudo et al published 3 studies examining data from the 15th (1997-1998) plus 16th (1999-2000) and the 19th (2005-2006) nationwide surveys in Japan including children treated with IVIG within 10 days; and giant CAA were defined as a diameter of ≥8 mm. Although the data on gender remained inconclusive, these studies found ‘young age’ (i.e. <1 year) and a ‘second IVIG dose’ to be significantly associated with an increased risk of developing giant CAA.

We should notice that direct comparison of these Japanese studies with Western studies is difficult because of the possible differences in clinical practice and data analysis. The current treatment regimen for KD in Japan consists of a single, high dose infusion of IVIG. Conversely, at the era of the before mentioned studies, not all children received IVIG or used the same IVIG dosing regimen due to the medical insurance system in Japan. This is in part caused by the use of scoring systems for IVIG resistance, which are not applicable to patients with a Western background. Finally, these studies defined giant CAA as an artery diameter of ≥8 mm. We used z-scores adjusted for BSA instead, which may be a better indicator of abnormality. Also, in previous studies patients with...
giant CAA were compared with all other KD patients irrespective their coronary status, whereas we compared patients with giant CAA separately to patient groups based on CAA-status to also explore differences between the CAA-groups. To our knowledge, this is the first study searching for risk factors for the development of giant CAA based on z-scores in a Western population.

Our data suggest that there is a dichotomy between patients with giant CAA versus the other patients with CAA. First of all, practically all patients with giant CAA were male, at an age that hormones cannot yet explain any of the differences among these subgroups with or without large CAA. Although other studies have also found a male predominance in patient with giant CAA (Supplemental Table 2), this was never as outspoken. The surprising finding that CD40 has been repeatedly indicated as a potential genetic risk allele in KD, may suggest that its ligand on T cells (i.e. CD40L) plays a role, being an X-linked gene and hence affecting the male disease condition more dominantly than in female patients. Secondly, patients with giant CAA received a second IVIG treatment far more often than all other CAA-positive patients, even when compared to patients with the larger medium-sized CAA. This implies that patients with giant CAA have a longer duration of fever, because a second IVIG-dose will be given for continued or recurrent fever, i.e. treatment-resistance. In 38% of all patients with giant CAA, patients received steroids after IVIG treatment, indicating that in a considerable proportion of children the duration of fever was truly extended. Whether this possible dichotomy – recognizing patients with giant CAA as a distinct subgroup – has a genetic base for the worst disease course in KD is unknown. Interestingly, our recent analysis of carotid intima-media thickness measurements performed in a large KD cohort also indicated that patients with giant CAA could be separated from the remainder of KD patients by their different behavior in progression in vascular wall changes over time.

The high percentage of unresponsiveness to IVIG in the giant CAA group asks for an improvement of primary therapy for severe KD cases. A recent meta-analysis showed that corticosteroids added to the initial gift of IVIG may have a beneficial effect. However, the favorable outcome was only found in Japanese studies included in the review and was not found in two studies conducted in the USA. Thus, more research is needed to search for better and/or new primary treatment possibilities.

Most of the morbidity and mortality caused by KD are linked to giant CAA. Results of a recent study showed that none of the giant CAA of ≥ 8 mm in their cohort regressed and ischemia event-free survival rates were 0.63 and 0.36 at 10 and 20 years, which was not altered by the implementation of IVIG therapy as standard care. These studies indicate the impact and severity, but also show that a group of children may develop giant CAA irrespective of IVIG administration. Moreover, in our study, the progressive nature in size of many of the giant CAA lesions over time was remarkable, which is in line with a recent study reporting on coronary artery change beyond 1 month after disease onset. This again reinforces that repeated echocardiography is necessary, also if CAA are not marked as ‘giant’ during the first echocardiography, and especially if fever did not resolve by IVIG (treatment refractoriness).

In our study >80% of our giant CAA group had giant CAA of the RCA, which seems in line with the results of a study from 1994. Findings of this study, mostly from the pre-IVIG era, showed that in 170 KD patients who had undergone myocardial revascularization, 70% had giant lesions in the RCA, 53% in the LAD and 45% in the LMCA. An explanation for the predilection for the larger lesions in the RCA is unclear to date. For instance, both sides of the coronary tree are considered to be of the same origin, making a predilection for 1 side unlikely. Whether increased vulnerability of the RCA may relate to differences in the systolic pressure waves through the coronary vasculature, or the movement of the right ventricle causing more traction on the RCA compared to the LMCA and LAD contribute, is unclear.

Limitations

We should acknowledge that patients were included if they visited our multidisciplinary outpatient clinic from January 1999, as long as they had all acute echocardiographies performed at our center. Because children with coronary abnormalities are more likely to have further follow-up, this explains the relatively high proportion of patients with giant CAA in our study cohort. Second, this was a retrospective study using clinical care data rather than using a fixed research protocol, thus some deviations from standard protocol occurred.

The number of patients with giant CAA in our single-center cohort was limited, resulting in odds ratios with relatively large confidence intervals. We choose to categorize patients, who never received IVIG, in the ‘no IVIG re-treatment’ group to compare a ‘second IVIG-treatment’ to all other categories. This may have overestimated the significance of a ‘second IVIG-treatment’.

We used the ‘Boston z-scores’ while multiple formulas are available. A recent study showed that although coronary artery measurements have high inter- and intra-rater agreement, these z-scores formulas differ at larger CAA dimensions, meaning some patients may have fallen into different categories for other formulas.

Conclusion

This study evaluating risk factors for the development of giant CAA showed that ‘male gender’, ‘no or late IVIG treatment’, ‘IVIG re-treatment’, as well as ‘age <1 year’ were significantly associated with giant CAA when compared to patients without enlargement. When comparing patient with giant CAA to patients with medium-sized CAA, ‘IVIG re-
treatment’ was still significantly different between the groups, suggesting a dichotomy between patients with giant, treatment-resistant CAA and responsive other patients in which male gender may be factored in as a very relevant genetic trait. Of the coronary arteries, the RCA was most often affected by a giant CAA. Most giant CAA continued to increase in size during the first 20-40 days after disease onset, indicating the need for repeated echocardiographies, especially if fever is not resolved.

Acknowledgements
We thank Maarten Biezeveld, pediatrician at the Onze Lieve Vrouwe Gasthuis, and Willemijn Breunis, pediatrician at the Academic Medical Center, for their help with the completion of the database.

References


Supporting information
Supporting information applicable for Chapter 7.

**SUPPLEMENTAL TABLE 1**
Affected coronary arteries of children with giant CAA

<table>
<thead>
<tr>
<th>Giants</th>
<th>Medium</th>
<th>Small</th>
<th>Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RCA/LCA/LAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>RCA/LCA/LAD</td>
<td></td>
<td>RCA</td>
</tr>
<tr>
<td>3</td>
<td>RCA/LCA/LAD</td>
<td></td>
<td>LCX</td>
</tr>
<tr>
<td>4</td>
<td>RCA/LCA/LAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RCA/LCA/LAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>RCA/LCA/LAD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>RCA/LAD</td>
<td>LCA</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>RCA/LAD</td>
<td>LCA</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>RCA/LAD</td>
<td>LCA</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>RCA/LAD</td>
<td>LCA</td>
<td>LCX</td>
</tr>
<tr>
<td>11</td>
<td>RCA/LAD</td>
<td>LCA</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>RCA/LCA</td>
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<td>LCX</td>
</tr>
<tr>
<td>13</td>
<td>RCA/LCA</td>
<td></td>
<td></td>
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<td>LCA</td>
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<td>LCA</td>
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<td>16</td>
<td>RCA</td>
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<tr>
<td>17</td>
<td>RCA</td>
<td></td>
<td></td>
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<tr>
<td>18</td>
<td>RCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>LAD</td>
<td>RCA/LCA</td>
<td>LCX</td>
</tr>
<tr>
<td>20</td>
<td>LAD</td>
<td>RCA/LCA</td>
<td>LCA</td>
</tr>
<tr>
<td>21</td>
<td>LCA</td>
<td></td>
<td>LCX</td>
</tr>
</tbody>
</table>

RCA = Right coronary artery, LCA = Left coronary artery, LAD = Left anterior descending artery, LCX = Left circumflex artery
### References


### Supplemental Table 2: Studies describing gender and giant CAA in KD patient cohorts

<table>
<thead>
<tr>
<th>Author</th>
<th>Definition of giant CAA</th>
<th>Inclusion criteria</th>
<th>Total nr of patients (M/F)</th>
<th>Nr of patients with giant CAA (M/F)</th>
<th>Nr of patients with small-medium CAA (M/F)</th>
<th>Nr of patients with dilations (M/F)</th>
<th>Nr of patients without enlargement (M/F)</th>
<th>Nr of patients with (non-giant) CAA and no enlargement (M/F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lin et al, 2015</td>
<td>&gt; 8 mm, 1 month after KD</td>
<td>KD patients in a tertiary medical center between 1990 and 2012.</td>
<td>1073 (664/409)</td>
<td>27 (3/4)</td>
<td>109 (113/54)</td>
<td>x</td>
<td>8/70 (50/51)</td>
<td>x</td>
</tr>
<tr>
<td>Kitano et al, 2014</td>
<td>≥ 8 mm, 1 month after KD</td>
<td>Consecutive KD patients of the Wakayama (Japan) survey between 1999 and 2012.</td>
<td>1415 (798/616)</td>
<td>6 (2/4)</td>
<td>40 (30/10)</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Sudo et al, 2010</td>
<td>≥ 8 mm, 1 month after KD</td>
<td>15th nationwide Japanese survey; KD patients visiting hospital before 10th day.</td>
<td>1811 (1088/723)</td>
<td>53 (17/36)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>27/50 (105/200)</td>
</tr>
<tr>
<td>Nakamura et al, 2004</td>
<td>≥ 8 mm, 1 month after KD</td>
<td>15th + 16th nationwide Japanese survey; KD patients visiting hospital before 10th day. All patients with giant CAA (cases) and all patients from the same hospital (controls).</td>
<td>1844 (1180/664)</td>
<td>105 (83/22)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>20/39 (74/160)</td>
</tr>
<tr>
<td>Yanagawa et al, 2001</td>
<td>Diameter not specified, giant CAA acute stage</td>
<td>15th nationwide Japanese survey; all KD patients.</td>
<td>12666 (7496/5087)</td>
<td>M: 0.6% F: 0.3%*</td>
<td>M: 3.7% F: 2.2%*</td>
<td>408 (284/124)</td>
<td>M: 17.2% F: 11.4%*</td>
<td>1277/714</td>
</tr>
<tr>
<td>Yanagawa et al, 1998</td>
<td>Diameter not specified, giant CAA acute stage</td>
<td>15th nationwide Japanese survey; all KD patients.</td>
<td>13311 (7239/6072)</td>
<td>100 (60/40)</td>
<td>1510 (1051/459)</td>
<td>x</td>
<td>1042 (818/223)</td>
<td>x</td>
</tr>
</tbody>
</table>

CAA = Coronary artery aneurysms, M = Male patients, F = Female patients. * Only percentages mentioned in the manuscript, exact numbers are calculated from the percentages.
Regression and complications of Kawasaki disease-related giant aneurysms


Pediatric Cardiology, 2017, Epub ahead of print
Chapter 8  Regression and complications of z-score based giant aneurysms in a Dutch cohort of Kawasaki disease patients

Background: Kawasaki disease (KD) is a pediatric vasculitis. Its main complication is the development of coronary artery aneurysms (CAA), with giant CAA at the end of the spectrum. We evaluated regression and event-free rates in a non-Asian cohort of patients with giant CAA using the current z-scores adjusted for body-surface area instead of absolute diameters.

Methods: KD patients with giant CAA (z-score ≥ 10) visiting our outpatient clinic between January 1999 and September 2015 were included. Patient characteristics and clinical details were extracted from medical records. Regression was defined as all coronary arteries having a z-score of ≤ 3. A major adverse event was defined as cardiac death, myocardial infarction, cardiogenic shock or any coronary intervention. Regression-free and event-free rates were calculated using the Kaplan-Meier method.

Results: We included 52 patients with giant CAA of which 45 had been monitored since the acute phase. The 1-year, 2-year and 5-year regression-free rates were 0.86, 0.78 and 0.65, respectively. The 5-year, 10-year and 15-year event-free rates were 0.79, 0.75 and 0.65, respectively. Four children whose CAA would not have been classified as ‘giant’ based on absolute diameters instead of z-scores, had experienced an event during follow-up.

Conclusions: We found a high percentage of children in whom the lumen of giant CAA completely normalized. Four children not classified as ‘giant’ based on absolute diameters with z-scores of ≥ 10, experienced a cardiac event. Hence, the use of z-scores seems to be justified.

Introduction
Kawasaki disease (KD) is a pediatric vasculitis of the medium-sized arteries. Although its exact origin is still unknown, it is thought to be caused by an infectious agent in genetically predisposed children. While the disease is self-limiting, complications occur, with coronary artery aneurysms (CAA) being the most important one. CAA develop in approximately 25% of all untreated patients, although the introduction of intravenous immunoglobulins (IVIG) as treatment has decreased this percentage substantially.

At the extreme end of the spectrum of CAA are giant CAA. In Asian cohorts giant CAA typically do not regress, but evidence is scarce in the current era of more standardized treatment protocols. Most studies use cut-offs based on absolute diameters instead of z-scores. Z-scores are preferred as they correct the absolute size in mm for body-surface area (BSA) of the child.

Persistence of giant CAA causes an increased chance of thrombosis within and perfusion abnormalities distal to the CAA, potentially inducing ischemia and myocardial infarction (MI). Also, the persistence of giant CAA requires lifelong administration of anticoagulation by low-molecular weight heparin or vitamin K antagonists.

In this study, we report on a cohort of patients with giant CAA based on z-scores, evaluating regression and cardiac complications.

Methods
Study population and data collection
The Academic Medical Centre in Amsterdam (The Netherlands) is a tertiary referral center for patients with KD. At our center, we have a multidisciplinary outpatient clinic for cardiologic and/or (immunologic) long-term follow-up. The cohort of KD patients at our center consists of patients who were admitted to our center during the acute phase and who are admitted to other hospitals during the acute phase and are referred to our center for follow-up.

Patients with KD visiting our outpatient clinic between January 1999 and September 2015 were eligible. Patients were included if they had giant CAA during the acute phase of the disease. For analyses purposes, patients who were missed during the acute phase and patients of whom no information of the acute phase was available were excluded.

We retrospectively extracted clinical details from the medical records, i.e.: gender, age at disease onset, (in)complete disease presentation, treatment with IVIG including the day of first IVIG treatment, IVIG re-treatment, treatment with steroids, aspirin and/or anticoagulation therapy and size of the CAA at initial presentation and during follow-up.
Coronary artery aneurysms
The CAA-status was taken from information in echocardiography reports. The highest z-score of the left main coronary artery, right coronary artery or left anterior descending artery within the first six weeks was chosen in order to specify CAA[4,6]. Giant CAA are defined by a z-score of ≥ 10[4]. When using the Japanese criteria based on absolute diameters, a giant CAA is defined as diameter of ≥ 8 mm².

Outcomes: regression and major adverse events
During follow-up z-scores based on echocardiography, coronary angiographies (CAG’s), Magnetic Resonance Imaging (MRI’s) or Computer Tomography (CT)-scans were calculated. Regression was defined as all coronary arteries having a z-score of ≤ 3. CAA were also considered to have regressed when only a dilation of the origin of the coronary arteries with a z-score of 3 to 3.5 was present.

A major adverse event was defined as: cardiac death, MI, cardiac arrest or cardiogenic shock, coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI).

Statistical analysis
Regression
We calculated the number of days until all coronary arteries were regressed as demonstrated by an imaging procedure. If regression did not occur, the follow-up time was calculated until the date of the last imaging procedure with a maximum of 15 years. A regression-free survival curve was then constructed by means of the Kaplan-Meier method.

Major adverse event
If imaging suggested ischemia or infarction without preceding clinical presentation, the time from diagnosis until the date of the imaging was calculated. Patients were censored if no event occurred; follow-up time was calculated until the last registered imaging procedure with a maximum of 15 years. We assessed the 5-, 10- and 15-year event-free survival using Kaplan-Meier analysis.

Results
Patient population
In total, 52 patients with giant CAA visited our outpatient clinic. In six patients KD was missed. These six patients (5 men, 1 woman) presented with cardiac complaints due to ischemia and/or MI based on thrombus formation in a CAA or stenosis proximal or distal to a CAA. None of the patients had signs of atherosclerosis or cardiac disease, making KD the most likely cause of their giant CAA. In one extra patient, no information about the acute phase of the disease was available.

The remaining 45 KD patients had echocardiographic examinations within 6 weeks after acute disease onset. Patient characteristics and clinical data are shown in Table 1. Only 18 (40%) children would have classified as having ‘giant’ CAA based on absolute diameters. All but one child had been treated with IVIG. Most of them were treated with 2 g/kg IVIG in a single gift. Two children received 400 mg/kg during 5 days, and in 4 children dosing had not been recorded. Apart from a second IVIG gift, 11 (25%) children received methylprednisolone, oral prednisone, or both successively. Four (10%) children subsequently received either anakinra (IL-1 inhibitor) or infliximab (TNF-α inhibitor) due to IVIG and corticosteroid resistance. All children received high-dose aspirin during the acute phase followed by the standard low dose aspirin. Twenty-four children received additional anticoagulants (low-molecular-weight heparin or vitamin K antagonists). Most children not receiving anticoagulants at any point, had KD in an earlier era. Three children received an additional platelet aggregation inhibitor.

In 13 (29%) children aspirin was discontinued after the CAA were considered to have regressed completely. They had received aspirin for a median of 17.3 months (Interquartile range, IQR 8.8-151.5 months). None of these children experienced a cardiac event during or after discontinuation of aspirin therapy.

Regression of giant CAA
The 1-year, 2-year and 5-year regression-free rates were 0.86, 0.78 and 0.65, respectively (Figure 1). Remarkably, in 4 children the giant CAA had completely normalized more than 5 years after the acute phase of the disease. Apart from the 6 children with giant CAA going...
into complete regression, the largest change in z-score was observed in the first 2 years with an additional 13 children showing regression to medium or small CAA (Figure 2).

Due to the small number of children and the accompanying lack of power, we could not perform multivariable regression analyses to identify predictors for the persistence of giant CAA. Strikingly, only 4 (9%) children were girls, of which 2 had completely regressed CAA. A total of 23 (50%) children were under the age of 1 during the acute disease, of which 4 regressed within the first and an additional 2 within the second year. Of the 22 children over the age of 1 during the acute disease, 2 regressed within a year and 1 other child within the first 2 years.

Of the 19 children receiving a second IVIG dose, 6 had completely regressed CAA in a median time of 2.9 years (IQR 1.3-4.4). Of the 11 children receiving subsequent steroid treatment, 5 had regressed in a median time of 4.0 year (IQR 1.2-4.6).

Of the 14 children with an original z-score of 10-15, 11 (80%) went into complete regression. Of the children with an original z-score of 15-20, only 4 (25%) out of 16, and for the children with an original z-score of >20, we observed that only 3 (20%) out of 15 completely regressed (Supplemental Figure 1).

**Major adverse event**

A total of 12 cardiac events or interventions took place after a median time of 0.17 years (range 0.02-13.58 years) (Figure 3). All events accompanied with clinical symptoms occurred within 5 months after the acute disease, interventions and subclinical events occurred later. All events happened in children with, at that time, non-regressed CAA. The 5-year, 10-year and 15-year adverse event-free rates were 0.79, 0.75 and 0.65, respectively. In 4 children who would not have classified for a giant CAA according to the absolute diameters, a serious cardiac event took place, although significantly more events occurred in children with giant CAA based on absolute diameters ($p=0.041$).

Two children died as a result of MI. Another 6 children experienced MI. At time of the ischemic event, 3 children were using vitamin K inhibitors or low-weight heparin, 3
stenoses were seen directly proximal or distal to the CAA. None of these children had completely regressed CAA. Five of the children with arterial stenoses had experienced MI. In Table 2, the distribution of patients with cardiac events and with stenosis is shown.

Discussion

In a large Dutch cohort of KD patients with giant CAA based on z-scores, visiting our outpatient clinic during a 15 year period, we calculated 1-year, 2-year and 5-year regression-free rates of 0.86, 0.78 and 0.65. The 5-year, 10-year and 15-year event-free rates were 0.79, 0.75 and 0.65. This is the first study solely evaluating patients with giant CAA based on z-scores in a Western population.

A study by Chih at al., following 27 children with giant CAA (≥8 mm) found that none completely regressed CAA. Five of the children with arterial stenoses had experienced MI. In Table 2, the distribution of patients with cardiac events and with stenosis is shown.

In a large Japanese study, Tsuda et al studying 245 patients with giant CAA (≥8 mm), found 10-, 20- and 30-year cardiac event-free survival rates of 64%, 48% and 36%, respectively. The aforementioned study by Chih et al found 10- and 20-year ischemia-free rates of 52% and 21%. Our study showed higher cardiac event-free rates, which could partly be the result of the use of z-scores instead of absolute diameter cut-off.
However, 4 children whose CAA would not have been classified as ‘giant’ based on absolute diameters, did experience an event (cardiac arrest, MI and cardiogenic shock), which suggests that z-scores are helpful in identifying patients at high risk. Also, all but one patient in our cohort received IVIG, improving outcome as IVIG was found to be an independent risk factor for major adverse cardiac events (MACE) in a recent study.

Most events happened within the first months after the acute phase. Yet, in 3 patients, echocardiography or MRI showed signs of a small MI or subclinical ischemia, 10 years or more after the acute disease. Although conventional coronary angiography is still the gold standard to assess coronary anatomy and possible stenosis, this technique is invasive and exposes the child to radiation. Low-dose CT angiography is becoming more widely available and decreased the radiation burden significantly. Using echocardiography or cMRI with additional adenosine stress-testing systolic function and flow reserve capacity can be evaluated, yet cMRI cannot be performed in young children without the use of anesthesia. The results of these 3 patients suggest that all children should be followed-up with regular intervals to assess vascular flow reserve capacity using a combination of these techniques as we proposed previously.

Although we only observed cardiac events in children with persisting CAA at that time, it is unlikely that regression of CAA to a normal diameter of the arterial lumen will eliminate all future cardiovascular risk. Even if the lumen has a normal diameter, the arterial wall is supposed to be damaged and unable to adequately dilate upon increased cardiac demand. In a recent study using optical coherence tomography, changes in the coronary artery wall structure, especially intimal hyperplasia, were seen in CAA but also in segments where the CAA had regressed. This study indicates that these patients require life-long follow-up, even if the arterial lumen has gone back to normal size. In 13 children of our cohort aspirin therapy was discontinued after the CAA was considered to have regressed. Regarding the remodeling, persistent damage and increased stiffness of the arterial wall years after the acute phase, it is questionable whether antiplatelet medication should ever be discontinued in patients with regressed giant CAA. However, none of the children in whom aspirin had been discontinued experienced an event. This is in concordance with the study by Friedman et al. who found that none of the patients with regressed CAA experienced MACE. Stenosis, a result of a remodeling process of the artery, only occurred in children with CAA that had not completely regressed. In summary, more research is necessary for definite recommendations regarding (life-long) aspirin therapy.

Limitations
We calculated z-scores from absolute diameters described in echocardiography, CAG-, MRI- or CT-reports. Since approximately half of the patients were admitted to other hospitals during the acute phase of the disease, pediatric cardiologists in other centers had generated many of the early echocardiographies. Echocardiography is known for its measurement uncertainty, which could have influenced the regression as well as the time-to-regression.

For the time-to-regression, we registered the time until the first imaging procedure that demonstrated normality of all coronary arteries. The precise time of regression can therefore not exactly be defined. Hence, the time-to-regression has to be considered as the maximum time in which regression took place.

As this was a retrospective study, imaging was not performed according to a set protocol. This means subclinical ischemia could have been missed in children if no suitable imaging technique was performed.

Conclusion
In a Dutch cohort of KD patients with giant CAA based on z-scores followed-up from the acute phase, the 1-year, 2-year and 5-year regression-free rates were 0.86, 0.78 and 0.65. The 5-year, 10-year and 15-year major adverse event-free rates were 0.79, 0.75 and 0.65, respectively. In 4 children whose CAA would not have classified as being ‘giant’ according to absolute diameters instead of z-scores, a cardiac event took place. Therefore, z-scores are suggested to be a more sensitive tool to decide on life-long regular follow-up of KD children.
References


Supporting information

SUPPLEMENTAL FIGURE 1 Kaplan-Meier estimates of regression-free survival of patients with giant CAA, subdivided based on highest-ever z-score
PART III

IMMUNITY IN KAWASAKI DISEASE
Performance of MRP8/14 and human neutrophil elastase to discriminate acute inflammatory disease in Kawasaki disease from invasive infection in childhood


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Manuscript in preparation
Chapter 9  Performance of MRP8/14 and human neutrophil elastase to discriminate acute inflammatory disease in Kawasaki disease from invasive infection in childhood

Introduction: Kawasaki disease (KD) is a systemic vasculitis of early childhood, mimicking various infectious diseases. Early diagnosis is important to reduce the risk of coronary artery aneurysms by timely treatment with intravenous immunoglobulins. In this study, two neutrophil-derived biomarkers, MRP8/14 and human neutrophil elastase (HNE), were evaluated as possible biomarkers to distinguish between KD and infectious diseases.

Methods: Subjects with acute KD and children suffering from well-characterized various culture-positive infectious diseases were selected. Elastase complexes (elastase-α1 anti-trypsin [HNE-α1AT complexes]) and MRP8/14 were measured using in-house assays. KD samples before and/or after IVIG treatment were compared with samples of infectious disease patients.

Results: A total of 45 KD patients and 38 infectious disease patients were included. We did not find a significant difference in median MRP8/14 and HNE-α1AT concentrations between both groups during the acute phase. In KD, both markers were significantly higher during the acute phase as compared to the convalescent phase, suggesting a correlation between blood concentration and disease activity.

Conclusion: Neutrophil-derived MRP8/14 or HNE-α1AT levels in acute KD were increased. Their levels were not different when compared to those measured in patients with proven infectious diseases. Hence, no evidence of these markers being suitable as single biomarker for KD was found.

Introduction
Current studies have proven that it might be possible to discriminate bacterial and viral disease from each other which may be a highly meaningful way for early recognition, diagnosis and treatment decisions, irrespective the definite microbial factor involved in the etiology of the acute infection\textsuperscript{1-3}. By discriminating one from the other category unnecessary treatments and diagnostic measures may be limited.

One of the close mimics of infectious disease at early childhood consists of a febrile vasculitis, known as Kawasaki disease (KD). It is essential to diagnose this pediatric vasculitis since coronary artery aneurysms (CAA) can be the most important complication that - if treated in time – can be largely avoided by early treatment with single high-dose intravenous immunoglobulins (IVIG) and oral aspirin, instead of antimicrobial medication. KD is characterized by prolonged fever and additional features including a rash, lymphadenopathy, conjunctival injection, and abnormalities of mucosae and extremities. Due to this symptomatology, it’s often first diagnosed as a viral or bacterial infection. Also, bacterial and viral pathogens are regularly found in patients with KD. Although the etiology of the disease is still unknown, the current paradigm is that it is caused by an (infectious) trigger in genetically predisposed children\textsuperscript{4}.

Unfortunately, no diagnostic laboratory test for KD is available to date. The diagnosis is based on the presence of clinical criteria, which makes it plausible that the disease is easily missed and misinterpreted as an infection of childhood.

MRP8/14 (S100A8/9) or calprotectin is found in the cytoplasm of neutrophils and monocytes and belongs to a family of calcium-binding proteins\textsuperscript{5}. Upon inflammation, it is released as a heterodimer into the extracellular space\textsuperscript{5}. It is increased in and has been used as a biomarker for various inflammatory diseases\textsuperscript{6,7}. Most in particular in patients with inflammatory bowel disease, MRP8/14 in feces is used for detection and as a sensitive indicator of disease activity\textsuperscript{8}. Human neutrophil elastase (HNE) is a, more commonly known, marker of neutrophil activation. An earlier study showed that HNE is increased in the acute phase of KD compared to afebrile healthy controls\textsuperscript{9}.

The aim of this study was to evaluate whether MRP8/14 and HNE may act as possible biomarkers for acute KD and to which extent they can be used to discriminate the acute inflammatory vasculitis of KD from invasive infections.

Methods
**KD Patients**
Subjects with KD, based on the criteria of the American Heart Association were recruited. All patients and/or their parents gave informed consent as approved by our hospitals ethical research board. Patients were included in the Academic Medical Centre as well as multiple participating hospitals in the Netherland. Medical records of KD patients...
were reviewed to collect clinical details: age at disease onset, treatment and re-treatment with IVIG, and/or steroids, and C-reactive protein (CRP) values before IVIG, determined for clinical purposes. From echocardiography reports, information on the presence of CAA was extracted. We defined CAA by worst-ever z-scores: CA-dimensions as standard deviation units normalized for basal surface area. CAA was defined as a coronary z score ≥ 2.5.

Blood sampling
Plasma samples were collected before and/or after IVIG treatment. Due to ethical restraints, blood sampling coincided with control blood sampling and was not performed at standardized times. All samples drawn within 14 days after the first day of fever were considered ‘during the acute phase’. After the acute phase, blood samples were drawn if patients visited our follow-up outpatient clinic.

Bacterial infection patients
Controls were recruited at the medical University of Graz as part of the EUCLIDS consortium (EU-childhood life-threatening infectious disease study). Patients were eligible when they had a suspected invasive infection with organ involvement and a positive culture. Patients were included if they had one of the following four infectious diseases: sepsis, meningitis, osteomyelitis or pneumonia. Serum samples that were collecting within the first two days after hospital admittance were included. Clinical details were recorded as part of the EUCLIDS project including CRP values, gender and age.

Reference values
Reference values were measured in 19 plasma and 31 serum samples of healthy blood donors for MRP8/14 and elastase, respectively.

Analysis of neutrophil activation markers
Analyses of serum/plasma elastase complexes and MRP8/14 was performed as follows:

Elastase complexes (elastase-α1 anti-trypsin complexes, EA) were measured by an ELISA that has been adapted from a previously described radioimmunoassay. All incubations were performed in 100 μl volume at room temperature (RT) while shaking except coating. All washes between incubation steps were done with PBS 0.02% Tween-20 using Elix 405 (Bio Tek Instruments, Winooski, VT, USA). Briefly, Maxisorp Nunc-immunoplate (Thermo Scientific; Waltham, MA, USA) were coated overnight at RT with a polyclonal rabbit anti-human neutrophil elastase antibody (1.5 μg/ml; Sanquin, Amsterdam, The Netherlands). Standard (20ng/ml, twofold diluted further) and plasma samples (diluted to 4%, followed by two 5-fold dilutions) were diluted in high-performance ELISA buffer (HPE; Sanquin, Amsterdam, The Netherlands) and incubated for 1 hour. Bound complexes were detected after 1 hour incubation with biotinylated monoclonal anti-α1-antitrypsin antibody AT-15 (0.5 μg/mL in HPE), followed by a 30-minute incubation with horseradish peroxidase–labelled streptavidin (1:2000). Peroxidase activity was visualized with 100 μg/ml 3,3’,5,5’-tetramethylbenzidine (TMB; Merck Chemicals, Darmstadt, Germany) in 0.11 M sodium acetate, pH 5.5, with 0.003% (v/v) H2O2. The reaction was stopped with 2M H2SO4 and absorbance was measured at 450/540 nm. Results are expressed in ng/mL by reference to a standard curve of normal human citrated plasma in which elastase complexes were generated by incubating with porcine elastase (final concentration 2 μg/mL; Sigma Zwijndrecht, The Netherlands) for 1 hour at RT.

MRP8/14 (myeloid related protein 8/14, calprotectin, S100A8/9) was measured in a new optimized ELISA assay developed at our own lab. All incubations were performed in 100 μl volume at RT while shaking, except the coating. All washes between incubation steps were done with PBS 0.02% Tween-20 using Elix 405 (Bio Tek Instruments). Anti-human MRP8/14 monoclonal mouse IgG1 antibody, clone 27E10 (BMA Biomedicals, Augst, Switzerland), diluted to a concentration of 0.5 μg/ml in 0.1 M Carbonate buffer, pH 9.6, were coated onto a 96-wells flat bottom Maxisorp Nunc-immunoplate (Thermo Scientific) by overnight incubation at RT. Serum pooled from 30 different healthy donors were used as the calibration curve and was diluted to a concentration of 8.5 ng/ml (v/v), followed by two-fold dilution steps in PTG buffer. The MRP8/14 concentration of the serum pool was determined by usage of a commercial MRP8/14 ELISA kit (Bühlmann, Schönenbuch, Switzerland). Plasma samples were diluted to 1% or 0.1% (v/v), followed by five-fold dilution steps in PTG. As a positive control, serum pool was spiked with 10 μg/ml recombinant MRP8/14 (Hycult Biotech, Plymouth Meeting, PA, USA) and diluted to 0.1% (v/v) in PTG. All samples were incubated on the MRP8/14 monoclonal antibodies coated plates at RT for 1 hour. MRP8/14 was detected by incubating the plates with 0.5 μg/ml (in PTG) biotinylated mouse monoclonal IgG1 anti-MRP8/14 antibody, clone S36.48 (BMA Biomedicals) for 60 minutes. After washing, plates were incubated with 0.01% (v/v) streptavidin conjugated to poly-horseradish peroxidase (poly-HRP) for 30 minutes. Peroxidase activity was visualized with 100 μg/ml 3,3’,5,5’-tetramethylbenzidine (TMB; Merck Chemicals) in 0.11 M sodium acetate, pH 5.5, with 0.003% (v/v) H2O2. The reaction was stopped with 2M H2SO4 and absorbance was measured at 450/540 nm. Results are expressed in μg/ml by reference of the calibration curve. All used concentrations were analysed and were determined to be the optimum concentrations for the determination of MRP8/14 concentration in human plasma.
**Statistics**

Demographic and clinical characteristics between groups were compared using Mann-Whitney U test of chi-square test. Acute samples before and after IVIG were compared to samples of patients with infectious disease patients using Mann-Whitney U tests. Paired samples before IVIG, during treatment and/or after treatment/during convalescence were compared using Wilcoxon rank test. During treatment was defined as after (the first) IVIG treatment but, in those with resistance to the first dose of IVIG (i.e. persistence of fever >38.5°C in the presence or absence of additional inflammatory symptoms or high CRP), prior to or during additional treatment. During convalescence was defined as no anti-inflammatory treatment except for aspirin in patients with CAA.

Graphs were constructed using graphpad software (version 7.02).

**Results**

**Study population**

A total of 45 KD patients and a total of 38 patients with acute bacterial sepsis (18), meningitis (9), osteomyelitis (3) and pneumonia (8) were included. The microbiological blood cultures of the included patients diagnosed with sepsis were mostly positive for *Neisseria meningitidis* (67%). Demographic and clinical characteristics of KD patients and children included in the infection cohorts are shown in Table 1. Median CRP of KD patients before IVIG was 115 mg/l (Interquartile range, IQR 59-173 mg/l). Median CRP of infectious disease patients was 99 mg/l (IQR 42-220 mg/l). There was no significant difference in CRP between the two (p=0.754).

**Diagnostic marker**

Samples from acute KD patients were compared with samples of patients admitted because of acute infectious disease. A total of 28 children had a blood sample taken before IVIG and 21 children had a blood sample taken after IVIG within the first 14 days after disease onset. Median MRP8/14 before IVIG was 569 ng/ml (IQR 347-1113 ng/ml), after IVIG was 627 ng/ml (IQR 142-957 ng/ml) and of patients with acute infectious diseases was 646 ng/ml (164-963 ng/ml). MRP8/14 before and after IVIG did not differ significantly from infectious disease patients (p=0.985 and p=0.605, respectively). Normal control values were below 25 ng/ml (95% cut-off of median of normal values). Median values for HNE-α1AT before and after IVIG administration were 617.5 ng/ml (IQR 393.8-1929.0 ng/ml) and 364.0 ng/ml (IQR 151.8-984.5 ng/ml), respectively, as compared to the median concentration in acute infectious disease patients of 872.5 ng/ml (IQR 295.8 ng/ml-1810 ng/ml, sepsis), respectively. There were no significant differences in HNE-α1AT complex measurements between KD patients and infectious disease patients (pre-IVIG vs infection, p=0.952; post-IVIG vs infection, p=0.06). Normal control values were below 25 ng/ml (95% cut-off of median of normal values).

**TABLE 1** Demographic and clinical characteristics of children admitted for Kawasaki disease or invasive infections

<table>
<thead>
<tr>
<th>CAA-status</th>
<th>KD patients N=45</th>
<th>Infectious disease N=38</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVIG re-treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- No enlargement</td>
<td>31 (68%)</td>
<td>22 (42%)</td>
<td>0.754</td>
</tr>
<tr>
<td>- Small-medium CAA</td>
<td>10 (21%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>- Giant CAA</td>
<td>4 (9%)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* At first blood sampling, median (IQR). ** Of the 28 samples taken before IVIG.

**Follow-up samples**

A total of 31 children had paired measurements before IVIG or during treatment for IVIG-resistance; and during convalescence (Figure 2). Samples collected prior to IVIG administration showed significantly higher levels of MRP8/14 compared to the samples following treatment during convalescence (p<0.001; n=25 paired samples). Median time after disease onset of the samples taken during convalescence was 44 days (IQR 23-89 days). Six samples were taken during treatment; 5 after the first but before the second IVIG gift, the last sample was taken after the second IVIG gift but before steroid treatment. These samples were also significantly higher compared to convalescence (p=0.23; n=6 paired samples). Median time of samples taken during convalescence was 32 days after disease onset (IQR 15-263 days). Although some samples still showed high MRP8/14 levels during convalescence, the majority of samples were in the same range as samples taken from 19 healthy controls.
PART III

of cardiac or coronary injury and genetic markers. Yet, most of these markers are not specific for the diagnosis of KD and do not differentiate between KD and other febrile illnesses. The symptomatology of KD resembles multiple (severe) infectious diseases. Treatment with IVIG is recommended to be given within the first 10 days after the onset of fever. Delayed treatment and no treatment is associated with the development of CAA. Differentiating between KD and severe infectious disease could possibly prevent the lack or delay in treatment with IVIG, as many children will first be treated with antibiotics and thus largely present the development of CAA.

MRP8/14 is also called S100A8/A9. This heterodimeric protein belongs to the family of S100 proteins, which have both intra- and extracellular regulatory effects. MRP8/14 is considered to function as a danger-associated molecular pattern molecule (DAMP). DAMPs are proteins that mediate immune responses through recruitment and activation of immune cells after being secreted. Although its exact role during the immune response is still rather unclear, MRP8/14 has been indicated to act as an acute phase reactant (being not synthesized in the liver) with a role in the inflammatory reactivity of the host. It can stimulate the secretion of pro-inflammatory cytokines, including TNF-α and IL-1β, by binding to Toll-like Receptor 4 (TLR4) and Receptor for Advanced Glycosylation End-products (RAGE).

For elastase, values before IVIG were significantly higher compared to during convalescence (p<0.001; n=25 paired samples). Concentrations during treatment were not significantly higher compared to during convalescence (p=0.563; n=6 paired samples).

Discussion

We evaluated MRP8/14 (S100A8/9) and elastase (HNE) during the acute phase of KD. Although both parameters were increased during the acute KD phase compared to the convalescent phase, neither of the parameters was significantly increased compared to patients with infectious disease. Hence, we found no evidence that these inflammatory markers can be used as biomarkers for KD.

Although KD was first described 50 years ago, up to date no diagnostic biomarker for KD is available. Multiple markers have been suggested including markers of inflammation,
Another drawback of the study is that the samples of KD patients were collected as EDTA plasma while samples of infectious disease patients were collected as serum. Although it is thought that calcium in MRP8/14 is bound tightly and remains complexed in the presence of EDTA in plasma, we further corroborated this by showing that EDTA at 10 and 20 mM did not prevent the MRP8/14 from being assessed at similar levels, demonstrating that EDTA does not hinder the measurement in plasma. This means that values should be comparable. Nonetheless, it is possible that the coagulation in serum causes neutrophil activation and higher levels of both MRP8/14 and HNE. One study measured plasma and serum samples of the same patients and found consistently higher MRP8/14 levels in serum\(^2\). It is thus possible that there is a difference in concentration between serum and plasma which may cause an underestimation of the (possible) difference in MRP8/14 and in HNE between the KD and infectious disease patients in our study. Studies using the exact same material is now being performed to appropriately address these issues.

As this was a small, hypothesis-generating study, we did not correct MRP8/14 and HNE samples before IVIG for time since disease onset. Nonetheless, since we only evaluated samples taken within the first 14 days after disease onset, we did not expect the data to fundamentally change.

**Conclusion**

In this study evaluating MRP8/14 and elastase in plasma of KD patients, we did not find increased levels of MRP8/14 nor HNE compared to patients with infectious diseases. Hence, we did not find evidence that these inflammatory markers are suitable as single biomarker for KD.

A few studies have previously reported on MRP8/14 levels in children with KD. Abe et al. found increased MRP8/14 levels in 32 KD patients before IVIG treatment compared to after IVIG treatment and compared to febrile controls\(^1\). Viemann et al. found that MRP8/14 concentrations dropped significantly within 24 hours after IVIG treatment in 21 KD patients\(^2\). After 1 month, the concentrations had reached the values of healthy controls. Remarkably, these authors also found that the endothelium of the coronary arteries from myocardial sections of 3 deceased KD patients were coated when with this protein when stained with antibodies against MRP 8 or 14. Hirono et al. found increased MRP8/14 concentrations before IVIG as compared to post-IVIG in 45 IVIG-responding KD patients, whereas levels did not decrease after IVIG in 16 IVIG non-responders\(^3\). Four weeks after IVIG treatment, these patients still had higher MRP8/14 concentrations compared to IVIG-responders. In these studies no patients admitted with definite infections were included as controls, which is the group of patients that KD need to be discriminated from during the acute phase. We found that MRP8/14 as a single biomarker could not discriminate between invasive infection and the inflammatory response of KD. We also were not able to correlate these values with CAA development or with IVIG response. Although some samples still had high values during convalescence, most were in the same range as samples taken from healthy controls, thus seemed to have normalized.

The exact location of MRP8/14 within neutrophils and the mechanism how neutrophils release this factor is still not fully elucidated\(^4\). In contrast, HNE is a well-studied serine protease stored in the cytoplasmic azurophilic granules of neutrophils. Upon inflammation it is secreted and has antimicrobial properties and plays a role in the innate immune response\(^5\).

Biezeveld et al. investigated HNE levels during the acute phase of KD in 28 patients and compared them to afebrile, healthy controls\(^6\). They found that HNE levels were significantly increased up to 3 months after the acute phase of KD. Takeshita et al. compared 15 acute KD patients to 7 patients with sepsis\(^7\). They did find increased neutrophil elastase levels in KD patients compared to the sepsis patients. In our larger study, we did not find a difference in HNE levels of KD patients compared with children with acute infectious diseases.

**Limitations**

Due to ethical restraints we did not have standardized measurements of all KD patients before IVIG and within days after IVIG administration. However, we did have a measurement at variable time points after IVIG from most patients included; hence we could compare these values with the corresponding pre-IVIG values at a more variable follow-up.
CHAPTER 9


References

PART IV

SUMMARY AND DISCUSSION
Summary, general discussion and future perspectives
Connecting the dots
Monitoring strategies and long-term consequences of Kawasaki disease

Chapter 1 gives an outline of this thesis. Chapter 2 is a narrative review concerning KD and together form an introduction to the topic. Different features of the disease are being discussed including the current hypothesis on (infectious) triggers for the disease, immunological pathways, genetic predisposition and current treatment options. Furthermore, the different methods by which CAA can be classified and the (long-term) consequences of CAA and the possible long-term consequences of KD are being discussed.

PART I – Cardiovascular risk and the follow-up of patients after Kawasaki disease

Part 1 of this thesis consists of 3 studies involving surrogate markers for cardiovascular disease in patients after KD. First, we conducted a systematic review evaluating studies examining flow-mediated dilation (FMD), stiffness index (SI), carotid intima-media thickness (cIMT), pulse wave velocity (PWV) and peripheral arterial tonometry (PAT), described in Chapter 3. We showed that studies were often small and of poor quality. Moreover, results of studies were very heterogeneous. Hence, most results from these published studies could not be pooled. Nonetheless, the majority of studies evaluating FMD found this parameter to be decreased in patients after KD, being more outspoken in patients with CAA. The majority of studies evaluating SI, found this to be increased in KD - and more specifically in the CAA-positive patient group. In studies measuring mean cIMT (across a distance of, usually, 1 cm), this was neither significantly increased in KD in general, nor in the CAA-positive group when pooling the data of those studies. Studies measuring maximal cIMT (across a distance of, usually, 1 cm) showed conflicting results. There were a limited amount of studies evaluating PAT and PWV, and for the latter, different methods were used in different studies. Hence, the results could not be combined.

cIMT represents the thickness of the intimal and medial layer of the carotid artery and is largely measured using ultrasonography. It is an validated surrogate marker for the risk of cardiovascular events, such as ischemic stroke and myocardial infarction. However, the very thin vascular layer is measured with techniques that may have a limited resolution, making the measurement subject to technical flaws and considerable variability between the different examiners. Although our systematic review for that reason was not able to not show cIMT to be an indicator for vascular disease in KD during follow-up, we performed a cross-sectional as well as a longitudinal follow-up study evaluating cIMT in patients.
over a large period of time during their convalescence from the age of 5 years onwards. For our cross-sectional study described in Chapter 4, we included 168 patients with a history of KD and 82 controls aged 7-20 years. We found that patients who had suffered from an episode of acute KD in the past had a significantly increased cIMT compared to controls (0.378±0.030 mm vs. 0.360±0.027 mm; P<0.0001). Because patients were measured at different ages, we could plot the difference in cIMT between patients and controls against age. We found that in CAA-negative patients, AIMT decreased with age and thus cIMT seemed to normalize in patients when they got older. In contrast, in CAA-positive patients, the difference between patients and controls remained.

With the results of the cross-sectional study in mind, we analysed our longitudinal follow-up data evaluating cIMT in 316 patients with a total of 528 measurements and 150 controls, the largest study ever performed in KD, as described in Chapter 5. The group of KD patients included 51 patients with small- to medium-sized CAA and 27 patients with giant CAA. We performed a multilevel, repeated-measures, linear-mixed-effects model. Through this model, it was possible to analyse all measurement, both of patients with multiple as well as of patients with single measurements.

We could conclude that the estimated marginal means for CAA-negative patients, patients with small-medium CAA and patients with giant CAA were significantly increased compared to controls (0.373 mm [95% CI 0.369-0.376 mm; P<0.01 compared to controls], 0.374 mm [95% CI 0.367-0.382 mm; P<0.05 compared to controls] and 0.381 mm [95% CI 0.370-0.392 mm; P<0.01 compared to controls], respectively). Furthermore, we found that CAA-negative patients started with a significantly higher cIMT compared to controls, but this difference decreased per year, normalizing to cIMT values measured in non-KD controls. Conversely, patients with giant CAA started with a (not-significantly) higher cIMT, but also had a trend towards and increased cIMT progression each year, potentially resulting in an increased cIMT at a later age.

Particularly the cIMT measures in CAA-negative patients which were initially increased but later on normalized, are a remarkable finding, since cIMT is being primarily used as an indicator of premature atherosclerosis. If KD indeed would cause premature atherosclerosis, it is unlikely that the cIMT will diminish without medication or a significant life-style intervention. Thus, in our patients it is unlikely that the increased cIMT in KD patients is caused by premature atherosclerosis. This is in line with studies which did not find any signs of atherosclerosis in the coronary arteries either on optical coherence tomography or intravascular ultrasound, nor by histopathology in any of the coronary artery specimens of deceased KD patients\(^{182,183}\). Nevertheless, another study did find plaques in regressed or persistent coronary segments with possible signs of atherosclerosis such as necrotic cores and fibrofatty components using virtual histology-intravenous ultrasound in patient with a median time of 15.9 years after KD\(^4\). Yet, no such findings of atherosclerotic abnormalities were found in normal coronary segments. Even if the plaques in diseased coronary arteries in KD patients could indeed be composed of atherogenic material, the coronary arteries without enlargement and not importantly involved in the former aneurysmatic lesions seemed unaffected by atherosclerotic changes. We may hence extrapolate that it is unlikely that the peripheral arteries in the body will be affected by the atherosclerotic changes as a feature of a more general vascular disease.

The two most important questions that remain, involve the origin of the “KD vasculopathy” and the actual meaning of the increased cIMT for the risk-development of the KD patients later in life.

A possible method to evaluate the composition or components of the thickened intima-media is carotid MRI\(^5,6\). However, as the cIMT increase in KD patients is small and the subsequent changes of the carotid wall minor, it is questionable whether the different components can be distinguished.

With respect to its development over time, the only method to truly evaluate cardiovascular consequences in KD patients are long-term follow-up studies. There are several studies evaluating KD patients with giant CAA up to 30 years after KD\(^7,8\). These studies are limited and mainly reflect the consequences of the most extreme part of the spectrum, being giant CAA (myocardial infarction due to thrombosis in a CAA or stenosis proximal or distal of a CAA) rather than the consequences of the vasculitis on the vascular system per se. Another drawback is that the majority of these patients with giant CAA were included into the studies decades ago; many have not been treated with IVIG during the acute phase as this was not yet the standard therapy in that era. Also long-term studies of patients without CAA remain necessary to determine whether KD causes a cardiovascular risk. At the same time, these studies will be challenging, as these patients may have no noticeable consequences or symptoms. It is therefore important to find a way to keep these patients into follow-up programs.

As many aspects of KD, including the long-term cardiovascular effects, are still largely unknown, the best way to follow these patients after KD has been a subject of controversy over the past years. In Chapter 6, we described the current guidelines of the American Heart Association (AHA) and the Japanese Circulation Society. Both guidelines have drawbacks, involving the aggressive nature of the proposed imaging techniques and the lack of a complete examination of the coronary tree in children without CAA on echocardiography. The AHA guidelines were last revised back in 2004. We discuss possible imaging techniques such as MRI, low-dose CT and CT calcium scoring. We propose an
imaging pathway in which in all patients the complete coronary tree is depicted at least ones using MRI and in which CAA-positive patients are follow-up using techniques with no or a relatively low radiation dose. Since the publication of this manuscript, we have increasingly gained experience with a low-dose CT scan. This technique is showing promising results, especially in depicting the coronary anatomy. Studies have to be performed comparing low-dose CT scanning to echocardiography, and ultimately, to the current gold standard of coronary anatomy: i.e. a conventional coronary angiography (CAG). The latter is an invasive technique and exposes the child to radiation. Therefore, ethically, it will be difficult to justify a study involving this technique if there is no clinical necessity for it. Future studies will thus have to be directed into comparing low-dose CT-scans to echocardiography in patients without CAA or with small CAA and into comparing low-dose CT-scans with CAG in patients with giant CAA or complex CAA in whom it is important to obtain detailed information on coronary anatomy, in particular when surgical options are being considered because of doubtful flow reserve due to obstructive coronary artery stenosis as a consequence of previous severe CAA. Hopefully, in the future, non-invasive techniques with no or a low-radiation dose can completely replace the more conventional CAG as an informative imaging technique.

PART II – Giant aneurysms

In Part II of this thesis, we describe two aspects of giant CAA: risk factors for the development of giant CAA and the regression and complications of giant CAA. CAA can be classified using different criteria. The Japanese criteria use absolute diameters is combinations with the age of the patient; a lumen diameter of >3 mm is abnormal in children under the age of 5, a lumen diameter of >4 mm is abnormal in children over the age of 5. The past couple of years has been apparent that z-scores, adjusted for height and weight of the patient may be a better indicator of abnormality9,10.

Multiple studies have already been performed attempting to find risk factors for the development of giant CAA in Japanese children with a CAA-diameter cut-off of ≥ 8 mm11,12. Also, risk factors for all-size CAA, based on Japanese criteria, on z-scores, or on hospital codes for CAA have been investigated in non-Japanese cohorts13-16. Among many other parameters, risk factors for developing CAA reported previously, included ‘age <1 year or >9 years’, ‘low plasma albumin’, ‘male gender’ and ‘IVIG refractoriness’. As no study had yet attempted to find risk factors solely for giant CAA as based on the currently used z-scores, we analysed our cohort of patients in Chapter 7. This cohort consisted of patients who had their complete cardiac follow-up from the acute phase onwards at our tertiary centre to limit the effect of potentially confounding factors due to different investigators, ultrasound machines, treatment protocols, etc. When patients with giant CAA (z-scores ≥ 10 at any time during KD) were compared with patients without enlargement, risk factors were: ‘no or delayed IVIG treatment’, ‘young age (<1 year)’ and ‘IVIG re-treatment’. Remarkably, when comparing patients with giant CAA to patients with medium-sized CAA (z-scores between 5-10), patients with giant CAA still received a second IVIG gift significantly more often, which may well reflect on-going disease. This could thus relate to the longer duration of fever (as this is the common reason for the second IVIG gift), and thus a longer time to develop CAA. Other hypotheses are that it is caused by a more extensive or different immune response in these children (due to genetics or due to a different trigger), an inherited or imprinted resistance to IVIG, or another as yet undefined subtype of coronary arteritis different from KD.

We believe that there might be an underlying genetic polymorphism, making these children prone to both IVIG refractoriness as well as developing these above mentioned giant CAA. These genes are not necessarily identical. Up to date, multiple candidate genes have been associated with the susceptibility to developing KD, among which are genes encoding inositol-trisphosphate 3-kinase- C (ITPKC), Fcγ receptor-2A (FCGR2A), ATP-binding cassette sub-family C member 4 (ABCC4), and genes of the vascular endothelial growth factor (VEGF) and transforming growth factor (TGF-β) pathways27-30.

Again, these candidate genes may not be the dominant genetic factors involved in giant CAA. The reason that we believe genes contributing to susceptibility and severity or outcome are different comes from the remarkable observation from our study that 20/21 patients with giant CAA were male. Several polymorphisms in CD40 have been found to be association with both KD susceptibility and CAA susceptibility in Asian cohorts and KD susceptibility in European-American cohorts31-34. The ligand of CD40, CD40L is also encoded by an X-linked gene and has consistently been suggested to be associated with KD35,36. Although it is likely to expect that additional X-linked traits will impact the male preponderance in giant CAA, studies evaluating differences in CD40L expression and CD40 interactions related to specific genotypes could be a valid approach to find out whether such a link to giant CAA formation may exist.

In Japan, multiple risk scores have been established to predict a high risk for the development of IVIG resistance and thus for CAA37-39. Unfortunately, these risk factors are not applicable to patients with a Western or more ethnic diverse background, due to a very low sensitivity39. No risk scores have yet been developed for these patients. By combining the results of studies aiming to find risk factors with markers for certain SNPs or gene expression associated with CAA in a certain population, it should be possible to select children with a high a priori risk for CAA. Future studies could be directed to finding these markers and combining them with risk factors.

Nevertheless, a high a priori risk should be followed by a more intensive follow-up...
and treatment regime. In our study, we found that giant CAA continued to increase in size up to 60 days after onset of fever. Regular echocardiography is thus needed to follow these abnormalities and to administer anticoagulation therapy timely, to prevent early ischemia or myocardial infarction. Whether the continuation in size is caused by an ongoing inflammatory reaction or is caused by pressure within a damaged coronary artery is unclear. Therefore, it remains uncertain whether additional anti-inflammatory therapy would be beneficial. A more intensive treatment regimen for children with a high risk for developing IVIG resistance and/or CAA has been the subject of debate over the past years. Adding corticosteroids to the initial treatment with IVIG showed a beneficial effect on the prevention of CAA in a meta-analysis. However, this effect was not found in the two studies evaluating a Western population. Future studies should be aimed at selecting children with a high risk for CAA and finding further treatment possibilities for both initial and rescue therapy.

In Chapter 8 we describe a study in which we monitored a cohort of patients for a median time of 6.9 years. We evaluated both regression and complications of giant CAA. Twelve patients experienced a cardiac event, as defined by cardiac death, myocardial infarction, cardiac arrest or cardiogenic shock, or coronary artery bypass grafting or percutaneous coronary intervention. Eight of these had a MI. Remarkably, in three children the ischemia had not been noticed clinically, but was only apparent upon echocardiography or MRI. This shows that ischemic symptoms in children may go unnoticed or can be accompanied by atypical symptoms which were not clinically recognized as cardiac of origin. Compared to other studies, a relatively high percentage of patients reached complete regression of their CAA. This study indicated the disease burden of CAA and the need for long-term follow up in these patients.

PART III – Immunity in Kawasaki disease

In Chapter 9 we describe our study evaluating two inflammatory markers in children with and after KD. MRP8/14 (S100A8/A9) is an inflammatory marker, also known as calprotectin. It is being used as a marker of disease severity in inflammatory bowel disease when measured in faeces. We have developed and optimized an in-house assay to measure MRP8/14. Human neutrophil elastase (HNE) is another marker of neutrophil activity, more commonly used compared to MRP8/14. In blood, it is often being measured in complex with its major plasmatic serine protease inhibitor α1-antitrypsin (α1-AT): i.e. HNE-α1-AT complexes.

We measured MRP8/14 and HNE in the blood of a total of 45 KD patients. Of these patients, 28 had blood samples taken before IVIG and 21 had blood samples taken after IVIG within the acute phase of the disease (first 14 days). As controls, 38 children with culture-proven bacterial infections were included. We found that both biomarkers were increased in KD compared to controls, but neither MRP8/14 nor HNE levels were significantly more elevated when compared to (paediatric) patients with a range of acute, serious diseases such as sepsis, pneumonia and meningitis. During treatment of KD the MRP8/14 levels were found to significantly decrease. We also did not find a difference in MRP8/14 or HNE between patients with or without CAA or with or without IVIG resistance.

Even today, KD is regularly missed due to its resemblance with various viral and bacterial infections. Up to date several biomarkers have been investigated, yet none have shown to be able to solely diagnose KD. A biomarker with sufficient discriminating ability between KD and infectious diseases would be very useful because such a set of biomarker(s) could potentially decrease the amount of missed patients and prevent the development of CAA by early treatment.

The function of MRP8/14 may be that of a so-called danger-associated molecular pattern (DAMP). It can bind to the innate pattern recognition receptor Toll-Like Receptor 4 (TLR-4) and thus trigger the secretion of pro-inflammatory cytokines and many other cellular responses in immune and no-immune cells that express TLR4. Additional (co) receptors may be involved and will need to be studied in more detail.

Even though we found no evidence that MRP8/14 can act as a biomarker for KD, it might still be interesting to measure MRP8/14 levels during convalescence to investigate whether there is evidence of continuing low-grade inflammation. Some studies have found increased high-sensitivity CRP years after the disease, yet others did not. Apart from a marker in inflammatory bowel disease and a marker of acute infectious diseases, MRP8/14 may also be increased in patients with cardiovascular disease. It is known that MRP8/14 is secreted by myeloid-derived cells, mainly by neutrophils and to a lesser extent by monocytes. However, MRP8/14 may be considered under chronic conditions to, although in much lower concentrations, also be induced by non-myeloid cardiovascular cells. In cardiovascular disease it has been described that MRP8/14 induces adhesion molecule and chemokine expression and thus play an active role in atherogenesis. This means that increased long-term values could potentially be a result of a continuing low-grade inflammation, but could also play an active role in the (vascular) changes observed following KD. In on-going studies these follow-up samples, the correlation of MRP8/14 and other biomarkers with clinical response and outcome in KD (including CAA formation), as well as the cellular aspects of MRP8/14 will be investigated in more detail.

PART IV – Summary, general discussion and future perspectives

In Chapter 10, we have summarized and elaborated on the findings of the studies in this thesis. All studies show that there may be (genetic or other) subgroups within KD. KD
may cause a vasculopathy which affects patients with giant CAA differently from patients without CAA. An increased cIMT is often caused by an (premature) atherosclerotic vasculopathy resulting from a continues low-grade inflammation. Patients with giant CAA are remarkably often boys and show a different immune response as is shown by their (non)-response to IVIG. Apart from the studies in this thesis, knowledge about genetic polymorphisms, mainly in genes with immunomodulatory functions, involved in susceptibility to KD, CAA formation and IVIG-resistance will increasingly accumulate. Future studies should be directed towards finding subgroups and by doing so, increasing knowledge about the aetiology and treatment of KD. If we can indicate patients with a high possibility of (giant) CAA and treat them appropriately, the consequences of KD can be kept to a minimum.

References
APPENDICES

DUTCH SUMMARY/ NEDERLANDSE SAMENVATTING

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LIST OF PUBLICATIONS

PHD PORTFOLIO

WORD OF THANKS

ABOUT THE AUTHOR
Dutch summary/ 
Nederlandse samenvatting

Strategieën voor het monitoren en lange termijn consequenties van de ziekte van Kawasaki

Samenvatting
De ziekte van Kawasaki, ofwel mucocutaan lymfekliersyndroom, is een vaatontsteking van de kinderleeftijd. Tachtig procent van alle kinderen waarbij de diagnose wordt gesteld is jonger dan vijf jaar. In de meeste gevallen is de ziekte eenmalig, in enkele gevallen krijgen kinderen een tweede episode, vrijwel altijd binnen één jaar. De ziekte wordt vastgesteld op basis van symptomen omdat er geen diagnostische (bloed)test beschikbaar is. Deze symptomen zijn koorts, huiduitslag, rode ogen, rode, gezwollen of vervelende mond en/of lippen, vergrote lymfeklieren en rode en/of vervelende hand- of voetpalmen. Niet alle symptomen hoeven (tegelijkertijd) aanwezig te zijn. Ondanks dat de ziekte al in 1967 voor het eerste door dr. Kawasaki werd beschreven, blijven er nog veel aspecten onduidelijk.

De introductie van deze thesis bestaat uit hoofdstuk 1 en 2. Hoofdstuk 1 geeft een overzicht van de inhoud van deze thesis weer. Hoofdstuk 2 is een introductie over de ziekte van Kawasaki. In dit hoofdstuk wordt de oorzaak van de ziekte van Kawasaki behandeld. Momenteel wordt aangenomen dat de ziekte wordt veroorzaakt door een (infectieuze) uitlokker bij kinderen die daar een genetische aanleg voor hebben. Verschillende mogelijke uitlopers en genetische ‘pathways’ worden besproken. Daarnaast worden ook de behandeling met intraveneuze immunoglobulines (IVIG), aspirine, en aanvullende anti-inflammatoire medicatie in het geval de patiënt niet (goed) reageert op de IVIG, uiteengezet. Vervolgens worden de belangrijkste complicatie, het ontstaan van verwijdingen aan de kransslagaders oftewel coronaire aneurysmata, behandeld. Deze aneurysmata ontstaan tijdens het acute ziektebeeld. Gelukkig is het percentage patiënten die aneurysmata ontwikkeld, sterk afgenomen sinds er standaard behandeld wordt met IVIG. Er wordt ingegaan op het vaststellen van de aneurysmata en op de gevolgen hiervan voor de patiënt. Als laatste worden de mogelijke gevolgen op het hart- en vaatstelstel en op het gedrag van patiënten behandeld.

Deel I van deze thesis behandelt de mogelijke cardiovasculaire gevolgen van de ziekte van Kawasaki. Hoofdstuk 3 is een systematisch overzicht van alle studies die surrogaat
Deelnemers verschillende leeftijden hadden werden de metingen over de tijd uitgezet. Hieruit bleek dat kinderen met KD zonder coronaire aneurysmata initieel een verhoogde cIMT hadden, maar dat dit normaliseerde naar de waardes van gezonde broertjes en zusjes tegen jongvolwassen leeftijd. Daarentegen lieten de waardes van patiënten met grote coronaire aneurysmata een trend zien richting een snellere toename van de cIMT ten opzichte van de controles.

In hoofdstuk 6 wordt de cardiale follow-up van patiënten na de ziekte van Kawasaki beschreven. De huidige richtlijnen van de American Heart Association en de Japanese Circulation Society worden weergegeven met de voor- en nadelen. De verschillende beeldvormingstechnieken zoals cardiale MRI, een CT–scan met verminderde stralingsbelasting en CT-scan om kalk in de vaten te detecteren worden uiteen gezet. Als laatste presenteren we een flowdiagram voor patiënten na de ziekte van Kawasaki met de verschillende beeldvormingstechnieken. De verschillende ‘paden’ van het flowdiagram zijn gebaseerd op de ontwikkeling van coronaire aneurysmata en de ernst hiervan.

Deel II van deze thesis bestaat uit twee studies bij patiënten met de meest gevaarlijke complicatie, namelijk grote coronaire aneurysmata (giant aneurysmata). Hoofdstuk 7 presenteert een onderzoek naar de risicofactoren voor giant aneurysmata. Voor deze studie is gekeken naar de karakteristieken van alle patiënten die de gehele cardiale follow-up, vanaf het moment van de ziekte van Kawasaki, in het AMC hebben gehad. Deze patiënten zijn verdeeld in 4 groepen: zonder aneurysmata, met kleine aneurysmata, met matige aneurysmata en met giant aneurysmata. Vervolgens is gekeken naar verschillen in karakteristieken tussen deze groepen. Het was opvallend dat 20 van de 21 patiënten met giant aneurysmata jongetjes waren. Verder bleek dat, vergeleken met patiënten zonder aneurysmata, patiënten vaker een leeftijd van onder de 1 tijdens de ziekte hadden en vaker voor een tweede keer behandeld waren met IVIG. Ook werden kinderen met giant aneurysmata vaker niet of pas laat behandeld met IVIG. Het niet of pas laat worden behandeld met is een bekende risicofactor voor het ontstaan van aneurysmata. Wanneer patiënten met giant aneurysmata werden vergeleken met patiënten met kleinere aneurysmata waren er nog steeds verschillen. Vermeektelen met patiënten met matige aneurysmata reageerden patiënten met giant aneurysmata vaker niet op IVIG. Naast de risicofactoren onderzochten we ook het ontstaan van de giant aneurysmata. Hieruit bleek dat in veel gevallen de afmeting van het aneurysma (of de z-score, gecorrigeerd voor lengte en gewicht) nog tot 60 dagen na het begin van de ziekte kon toenemen. Het is daarom belangrijk om bij kinderen met aneurysmata frequent echo’s van het hart te maken.

Hoofdstuk 8 beschrijft alle kinderen met giant aneurysmata die de politikliniek van het AMC hebben bezocht in de afgelopen 15 jaar. In totaal kwamen 52 patiënten naar de

markers voor cardiovasculair risico in Kawasaki patiënten hebben bestudeerd. Surrogata markers zijn metingen die het risico op een ziekte kunnen voorspellen. Omdat de ziekte van Kawasaki nog relatief jong is, zijn de meeste patiënten nog niet op een leeftijd waarop hart- en vaatziekten te verwachten zijn. Onderzoek naar een eventueel verhoogd cardiovasculair risico door de vaatontsteking is dus alleen mogelijk met behulp van surrogata markers. In dit hoofdstuk worden studies naar 5 surrogata markers bij patiënten na de ziekte van Kawasaki en gezonde controles besproken: 1. Flow-mediated dilation (FMD): het vermogen van de arm slagader om goed te verwijden. 2. Stiffness index (SI): de stijfheid van de vaten. 3. Carotid intima-media thickness (cIMT): de dikte van de wand van de halsslagader. 4. Peripheral arterial tonometry: het vermogen van de kleine vaten in de wijsvinger om goed te verwijden. 5. Pulse-wave velocity: hoe snel stroomt het bloed van het ene naar het andere bloedvat. Helaas bleken veel van de studies weinig patiënten te hebben en bleek te kwaliteit van de meeste studies van mindere waarde te zijn. Daarnaast bleken er veel verschillen tussen de resultaten van de studies te zitten. De meeste studies die naar FMD keken concludeerden dat deze verlaagd ofwel slechter was in patiënten na de ziekte van Kawasaki en vooral bij patiënten die coronaire aneurysmata hadden ontwikkeld. Ook de SI was verhoogd en daarmee slechter in de studies bij patiënten met-, maar niet bij patiënten zonder coronaire aneurysmata. De gemiddelde cIMT was niet verhoogd, maar studies die keken naar de maximale cIMT vonden conflicterende resultaten. Er waren maar een paar studies die PAT en PWV hebben onderzocht bij kinderen na de ziekte van Kawasaki.

Na het systematische overzicht van de literatuur, hebben wij de data van cIMT, zoals gemeten bij patiënten in het AMC, na de ziekte van Kawasaki, geanalyseerd. **Hoofdstuk 4** is een cross-sectionele studie van cIMT metingen, gemeten tussen 2008 en 2013. Van alle 164 patiënten werd één meting genomen en van 82 onaangedane broertjes en zusjes werd de cIMT gemeten als ‘normaalwaarde’. De cIMT van kinderen na de ziekte van Kawasaki en gezonde controles besproken: 1. Flow-mediated dilation (FMD): het vermogen van de arm slagader om goed te verwijden, 2. Stiffness index (SI): de stijfheid van de vaten, 3. Carotid intima-media thickness (cIMT): de dikte van de wand van de halsslagader, 4. Peripheral arterial tonometry: het vermogen van de kleine vaten in de wijsvinger om goed te verwijden, 5. Pulse-wave velocity: hoe snel stroomt het bloed van het ene naar het andere bloedvat. Helaas bleken veel van de studies weinig patiënten te hebben en bleek de kwaliteit van de meeste studies van mindere waarde te zijn. Daarnaast bleken er veel verschillen tussen de resultaten van de studies te zitten. De meeste studies die naar FMD keken concludeerden dat deze verlaagd ofwel slechter was in patiënten na de ziekte van Kawasaki en vooral bij patiënten die coronaire aneurysmata hadden ontwikkeld. Ook de SI was verhoogd en daarmee slechter in de studies bij patiënten met-, maar niet bij patiënten zonder coronaire aneurysmata. De gemiddelde cIMT was niet verhoogd, maar studies die keken naar de maximale cIMT vonden conflicterende resultaten. Er waren maar een paar studies die PAT en PWV hebben onderzocht bij kinderen na de ziekte van Kawasaki.

Om deze initiële bevindingen te controleren zijn alle metingen van 2001 t/m mei 2015 geanalyseerd. De resultaten van deze follow-up studie staan in **hoofdstuk 5**. In deze studie zijn meerdere metingen per patiënt gebruikt. In totaal bestaat de studie uit 319 patiënten met 528 metingen. De groep van gezonde broertjes en zusjes werd uitgebreid tot 150. Opnieuw kon geconcludeerd worden dat de cIMT verhoogd was in de groep patiënten na de ziekte van Kawasaki ten opzichte van de controles. Daarnaast hadden kinderen met grote coronaire aneurysmata een dikkere cIMT dan kinderen zonder coronaire aneurysmata. Patiënten zonder coronaire aneurysmata hadden een verhoogde cIMT op de leeftijd van vijf jaar, maar vervolgens een minder snel toenemende dikte ten opzichte van de controles; de waardes normaliseerden zich ook tegen jongvolwassen leeftijd. Daarentegen lieten de waardes van patiënten met grote coronaire aneurysmata een trend zien richting een snellere toename van de cIMT ten opzichte van de controles.

In **hoofdstuk 6** wordt de cardiale follow-up van patiënten na de ziekte van Kawasaki beschreven. De huidige richtlijnen van de American Heart Association en de Japanese Circulation Society worden weergegeven met de voor- en nadelen. De verschillende beeldvormingstechnieken zoals cardiale MRI, een CT–scan met verminderde stralingsbelasting en CT-scan om kalk in de vaten te detecteren worden uiteen gezet. Als laatste presenteren we een flowdiagram voor patiënten na de ziekte van Kawasaki met de verschillende beeldvormingstechnieken. De verschillende ‘paden’ van het flowdiagram zijn gebaseerd op de ontwikkeling van coronaire aneurysmata en de ernst hiervan.
polikliniek waarvan 45 vervolgd waren vanaf de start van de ziekte van Kawasaki. Bij de andere patiënten was de ziekte van Kawasaki niet herkend, en deze presenteerden zich in het ziekenhuis vanwege klachten van het hart. Bij de patiënten die vanaf het begin vervolgd waren analyseerden we de complicaties en het in regressie gaan van de giant aneurysmata. In totaal kregen 12 kinderen ten gevolge van het giant aneurysma, te maken met een cardiale aandoening; een myocard infarct, een hartstilstand, hartfalen of een bypassoperatie. Een groot deel van de aneurysmata ging in regressie; het lumen van het bloedvat kreeg weer een normale diameter. De kans hierop leek kleiner wanneer het aneurysma in de acute fase groter was. Een aantal kinderen ontwikkelde een stenose; een vernauwing van het bloedvat. Meestal ontstond dit vlak voor of vlak na een aneurysma.

Deel III van deze thesis beschrijft één studie die gekeken heeft naar infectieparameters bij patiënten met de ziekte van Kawasaki. Hoofdstuk 9 is een studie waarin twee infectiewaardes in het bloed werden geanalyseerd: MRP8/14 en elastase. Beide waardes zijn gemeten in het plasma van patiënten met de ziekte van Kawasaki en bij patiënten met (ernstige) infectieziekten zoals bloedvergiftiging en longontsteking. Er was geen verschil in de parameters tussen kinderen met de ziekte van Kawasaki en met infectieziekten. We vonden dus geen bewijs dat een van beide parameters gebruikt kan worden als marker om de ziekte van Kawasaki te diagnosticeren.

Deel IV van deze thesis is een samenvatting en een discussie. De betekenis en mogelijke vervolgstudies van de verschillende hoofdstukken worden hierin beschreven.

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List of publications

International publications – this thesis


Dietz SM, Tacke CEA, Hutten BA, Kuijpers TW. Peripheral endothelial (dys)function, arterial stiffness and carotid intima-media thickness in patients after Kawasaki disease: a systematic review and meta-analyses, PLoS ONE 2015, 10 (7): e0130913

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Prasad M, Shenton P, Dietz SM, Saroha V, Whitehouse WP. What is the easier and more reliable dose calculation for iv Phenytoin in children at risk of developing convulsive status epilepticus, 18 mg/kg or 20 mg/kg? BMC Pediatrics 2013, 13(1):60.
PHD Portfolio

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PhD period: April 2013 – March 2017
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PHD training

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- Five years of Kawasaki disease in The Netherlands: a national surveillance study, Amsterdam Paediatric Symposium, Amsterdam
- Carotid intima-media thickness in patients after Kawasaki disease, IKDGS, Florence, Italy
- Carotid intima-media thickness in patients after Kawasaki disease, 11th International Kawasaki disease symposium, Honolulu, Hawaii, USA
- IVIG bij de ziekte van Kawasaki, Sanquin research night, Amsterdam
- Carotid intima-media thickness in patients after Kawasaki disease, Amsterdam Paediatric Symposium, Amsterdam
- Aetiology, course and long term effects of Kawasaki disease
- Dutch paediatric cardiology division
- Cardiovascular risk after Kawasaki disease
- Dutch paediatric immunology division
- Extracardial vasculopathy after Kawasaki disease
- Amsterdam Paediatric Symposium, Amsterdam
- Extracardial vasculopathy after Kawasaki disease
- Dutch Paediatric Society congress
- Kawasaki disease, presentation in Reinier de Graaf Gasthuis, Groene Hart ziekenhuis, LUMC, St Antoniusziekenhuis (paediatric and microbiology Department), Maassstadziekenhuis, Slotervaart ziekenhuis, Catharina ziekenhuis, Amstelland ziekenhuis 2013-2015 1.0
Supervising
Jeffrey Koole, bachelor thesis: “Risk factors for persistence of coronary artery aneurysms in children with Kawasaki disease: a retrospective cohort study”  2015-2016    1.0

Awards
“Insights into imaging most downloaded paper award 2016: Cardiovascular imaging in children and adults following Kawasaki disease ”

Posters (not presented)
Regression and complications of z-score based giant aneurysms in a Dutch cohort of Kawasaki disease patients
The annual meeting of the Association for European paediatric and congenital cardiology, Lyon, France
Giant aneurysms: a gender-specific complication of Kawasaki disease?
The annual meeting of the Association for European paediatric and congenital cardiology, Lyon, France

(A)National conferences
Amsterdam Paediatric Symposium, Amsterdam
Meeting IKDGS, Florence, Italy
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Meeting IKDGS, Honolulu, Hawaii, USA

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About the author

Sanne Marieke Dietz was born on the 2nd of October 1986. She grew up in Amsterdam where she attended primary and secondary school. After graduating in 2004, she stayed in Moratuwa, Sri Lanka for 10 months to work as a volunteer at a school for deaf children.

After returning to the Netherlands, she started her Medical studies at the University of Amsterdam. In 2009, she did her scientific internship researching syncope in children at the Queens Medical Centre in Nottingham, UK, under the supervision of dr. Whitehouse and prof. Vyas.

In 2012 she graduated cum laude from her medical studies after 2 years of clinical rotations. During her clinical rotations she participated in research involving the cardiovascular consequences of anorexia nervosa.

Directly after graduating, she started working at the Zaans Medisch Centrum as a paediatric resident as well as an emergency doctor for neurology, ophthalmology and urology.

In April 2013, she started working as a researcher at the department of paediatric haematology, immunology and infectious diseases at the Emma Children’s hospital. For almost 4 years, she worked on her research project on “Monitoring strategies and long-term consequences of Kawasaki disease” under the supervision of prof. T.W. Kuijpers. During these years she went to Hawaii to present part of the research at the 11th International Kawasaki Disease Symposium and she co-organised the Amsterdam Kindersymposium in 2015 and 2016.

Sanne lives in Amsterdam with Ian and their daughter Sofia.