Abstract

Background:
Coronary artery ectasia (CAE) is defined as a diffuse dilatation of the epicardial coronary arteries exceeding 1.5 folds the diameter of the normal adjacent arterial segment and/or the remaining non-dilated part of the same artery [1]. The incidence of CAE has been variably reported between different nations and ranges between 1.4 -10 % [2-5]. This wide range of variability is related to many factors including diverse definition of CAE, geographical distribution, association with other conditions (i.e. inflammatory, congenital or atherosclerosis) hence the existent uncertainty of disease burden and prevalence [6]. The main pathophysiology of CAE is initially understood to be part of atherosclerosis [3], yet others reported the non-atherosclerotic nature of the disease [2, 7]. The exact disease pathophysiology, prognosis and clinical outcome are not well studied; particularly the isolated, non-atherosclerotic form of the disease has not been fully determined or well identified.

Methods:
In paper one, we examined the clinical presentation, prevalence and cardiovascular risk profile of the CAE patients in acute myocardial infarction (MI). We investigated the inflammatory response and short-term outcome in CAE patients of 3,321 acute consecutive MI patients who underwent primary Percutaneous Coronary Intervention (PPCI) in two different centres in the United Kingdom (Royal Free Hospital, London and Norfolk and Norwich University Hospital) between January 2009 and August 2012.

In paper two, we studied the personalised lipid profile in 16 CAE patients from two different western European centres; Umea in Sweden and Letterkenny in Ireland (mean age 64.9 ± 7.3 years, 6 female). The lipidomic profile was compared with 26-control group (mean age 59.2 ± 6.6, 7 female) with normal coronary arteries.
In paper three, the immune-inflammatory response of CAE was examined, where the plasma levels of 16 CAE (mean age 64.9 ± 7.3 years, 6 female) were compared with 69 age and gender matched (mean age 64.5 ± 8.7 years, 41 female) subjects with evidence of coronary artery disease and 140 controls with normal coronary arteries (mean age 58.6 ± 4.1 years, 81 female). This expressed the cytokines response that is specific for CAE patients.

In paper four, we examined long term follow up data of CAE patients without atherosclerotic burden. This represents follow up data of 66 patients with CAE, among 16,464 patients, who underwent diagnostic coronary angiography in Umea, Sweden and Letterkenny university Hospital, Ireland between 2003 and 2009. Of the 66 patients, long-term follow up (mean 11.3 ± 1.6 years) data was complete in 41 patients (age 66 ± 9 years), 12 (27 %) Female, in the follow up period up to – and included- December 2018. All hospital readmissions with Major Acute Cardiac Events (MACE) including mortality, morbidity and hospital readmissions for acute coronary syndrome (ACS) were compared with gender matched 41 controls. No subject had >20% coronary stenosis in any coronary branch.

**Results:**

**Paper One:** The prevalence of CAE with acute MI was 2.7%. Apart from diabetes mellitus (DM), that was significantly less common in the CAE group (p=0.02), the other conventional cardiovascular risk factors were similar between ectatic and non-ectatic coronary arteries. The Right Coronary artery (RCA) and Left Circumflex artery (LCx) were predominantly involved in patients with CAE (p=0.001 and p=0.0001, respectively). CRP was higher (p=0.006) in the CAE group. While, total WCC, neutrophil and neutrophil/lymphocyte ratio (N/L ratio) were lower in the CAE group (p=0.002, 0.002 and 0.032 respectively), the localized form of the CAE has more prominent inflammatory response when compared with diffuse CAE (p=0.02, 0.008 and 0.03, respectively). The short-term follow-up of 2 years revealed no association between the acute inflammatory markers and MACE outcomes in
CAE (8/28, 28.6%) vs. the non-CAE group (13/60, 21.7%, p=0.42).

**Paper Two:** We identified 65 different metabolites between the CAE group and controls, 27 of them were identifiable using metabolomics library software (15 were fully identified and 12 were identified through the size of the side chains). Those metabolites showed sixteen species of phosphatidylcholines (PC); and 11 sphingomyelins (SM) species that all had significantly lower intensities in patients with CAE.

**Paper Three:** Systemic levels of IFN-γ, TNF-α, IL-1β, IL-6 and IL-8 were significantly higher in the CAE group compared to the CAD group (p=0.006, 0.001, 0.001, 0.046 and 0.009, respectively) and the control group (p=0.032, 0.002, 0.001, 0.049 and 0.007 in the same order). The levels of IL-2 and IL-4 were lower (p<0.001 for both) compared to the CAD and the control group. No differences were detected in the systemic levels of cytokines IL-10, IL-12P “subunits IL-12 and IL-23”, and IL-13 between the two patient groups.

**Paper Four:** The overall mortality and cardiovascular mortality was higher in the CAE group (p<0.001 and p<0.03, respectively) when compared with controls. MACE was similar in both groups (p=0.18), but females had more MACE than males (p<0.03). While CAE patients were slightly older, they were monitored for a longer period (p<0.001) than the control group. More patients in the CAE group smoked (p<0.001) and have a family history for CAD (p<0.02) when compared with the controls, but these variables were not different between survivals (36 patients) and non-survivors (5 patients). Finally, all non-survivors and 12/36 survivors had smoked and had dyslipidemia.

**Conclusion:**

Coronary artery ectasia, despite of common association with atherosclerosis, had a lower disease prevalence and dysregulated lipid metabolic profile than atherosclerosis. The pro-systemic inflammatory response in CAE is different from atherosclerosis with different Cytokines milieu. In the context of CAE with acute myocardial infarction, the management
options should follow the standard guidelines for revascularization. CAE may lead to exaggerated inflammatory response in the acute settings but the short-term outcome is similar to non-ectatic obstructive CAD and the inflammatory markers levels had no different prognostic values in management. However, long term follow up data showed CAE may have worse prognosis with higher mortalities and trends to higher MACE rates in females, yet no statistical difference was found in overall MACE rate between both groups.

List of papers:


Abbreviations

Acute coronary syndrome: ACS
Cardiovascular: CV
Coronary artery bypass graft: CABG
Coronary artery disease: CAD
Coronary artery ectasia: CAE
Coronary slow flow: CSF
C-Reactive protein: CRP
Diabetes mellitus: DM
Ectatic infarct-related artery: EIRA
Electrocardiogram: ECG
Endothelial cells: ECs
Enzyme linked immunosorbent assay: ELISA
Frame count method: TFC
Glycoprotein IIb/IIIa inhibitors: GPI
Interferon: IFN
Interleukin: IL
Intra-vascular ultrasound: IVUS
Ischemic heart disease: IHD
Left anterior descending artery: LAD
Left circumflex artery: LCX
Light chromatography-mass spectrometry: LC-MS
Low density lipoprotein: LDL
Lowest detection limit: LLOD
Major acute cardiac events: MACE
Mass feature extraction: MFE
Matrix metalloproteinases: MMPs
Myocardial infarction: MI
Neutrophil/lymphocytes ratio: N/L ratio
Oxidized- LDL: ox-LDL
Percutaneous coronary intervention: PCI
Primary Percutaneous coronary intervention: PPCI
Phosphatidylcholines: PC
Principal components analysis: PCA
Projection to latent structure - discriminant analysis: PLS-DA
Right coronary artery: RCA
Smooth muscle cells: SMC
Sphyngomyelins: SM
ST elevation myocardial infarction: STEMI
Thrombolysis in myocardial infarction: TIMI
T-helper cell 1/2: TH1 and TH2
Takayasu arteritis TA
White cell count: WCC
Insights into Coronary Artery Ectasia

Introduction

Epidemiology of coronary artery disease

Coronary artery disease (CAD) is a major cause of death and disability in the world[8-10], owing to the increasing prevalence of myocardial infarction (MI) in the general population [11]. Age-adjusted rate to describe CAD related-mortality masks the true estimation of CAD as a primary cause of death in younger population. The increasing CAD burden is proportionally parallel to the decrease in life expectancy [12, 13]. In Europe, CAD is responsible for more than 45% of all deaths and remains one of the leading causes of death [14]. Furthermore, in the USA, 35% of total deaths in the entire population is related to CAD [15]. Conventional cardiovascular (CV) risk factors are well established and often correlate with MI, especially in women. Although risk factors predictive value for recurrent ischemic events is marginal in men, a strong associations exist in women [16].

Pathophysiology of Coronary artery disease

The core concept of the pathogenesis of atherosclerosis is attributed to chronic inflammatory processes, which is initiated by the oxidation of low-density lipoproteins (LDL), forming oxidized LDL (ox-LDL). This trigger disproportionate increase in fibrosis of the intima, the formation of fatty plaques and the proliferation of smooth muscle cells, which are preceded by the migration of an assemblage of cells, mainly monocytes and T cells. This leads to the activation of macrophages into the T-helper cells 1 (TH1) pathway in response to inflammation [17, 18].
Therefore, progression of atherosclerosis comprises the following two main mechanisms; the accumulation of fat engulfed by activated macrophages, followed by sclerosis enriched by the fibrosis layer of smooth muscle cells (SMC), leukocytes, and connective tissue [18]. Atherosclerotic plaques grow with the proliferating fibrous tissue and smooth muscle, consequently reducing the intraluminal diameter compromising the blood flow. Finally, fibroblasts and calcium deposition in the lesion results in hardening of the arteries, the creation of uneven surfaces, thrombus formation and sudden arterial blockage leading to MI [19]. This process is enhanced by immune-inflammatory cytokines as they modulate endothelial cell (ECs) permeability and hence fatty streak deposition [20]. Both of Interferon IFN-γ and tumour necrosis factor TNF-α cause the restructuring of the actin and tubulin cytoskeletons in ECs, thus forming gaps between adjacent cells. This is a prominent process of endothelial damage that is mediated by cytokine activation and immune-inflammation [21].

Conventional CV risk factors such as hyperlipidaemia, hypertension, and hyperglycaemia are related to increased oxidative damage and lipoprotein levels increasing 10 years CV risk profile [22, 23]. These correlations are paradoxical with insulin dependent DM. At the level of lipid metabolite analysis (known recently as Personalized lipid profiling “Lipidomics”), the plaque analysis confirmed the presence of polyunsaturated cholesteryl esters with long-chain fatty acids and sphingomyelin (SM) species plaques [24]. SMs are carried into the arterial wall by atherogenic lipoproteins. They stimulate lipoprotein aggregation and macrophage foam cell formation, contributing predominantly to plaque formation. The levels of SMs has been shown to be independently and directly predictive of the co-existence and progression of atherosclerotic CAD [25].
Coronary Artery Ectasia - CAE

A) Definition of coronary artery ectasia

CAE is defined as dilatation of an arterial segments to a diameter at least 1.5 times that of an adjacent normal artery and involves more than one third of the length of the artery [26-30]. However, a strict definition of ectasia based on a dilated segment length more than 20 mm has been proposed [31]. The first definition is widely accepted as it includes a diffuse involvement in the ectatic segment. This is to avoid the misrepresented definition of CAE as a localized aneurysmal dilatation restricted to a short segment (i.e. localized) of the artery that occurs predominantly secondary to atherosclerosis [32]. The anatomy and location of the affected artery is commonly confined to the right coronary artery (RCA), while in atherosclerotic coronary disease causing aneurysmal dilatation is predominantly confined to the left anterior descending artery LAD [6, 32, 33].

B) Epidemiology of CAE and disease burden

The prevalence of CAE varies between 1.5- 5% of all patients who underwent coronary angiography for ischemic heart disease (IHD) risk stratification [26, 28-31]. However, the conflicting data about the true prevalence of CAE has reported a wide range between 16 % [30] and as low as 1.05% [29]. Nevertheless, CAE was documented in 1.4% of autopsy successions [34]. Despite of, a lower incidence of CAE was reported in a more recent study, that may reflect the strict adherence to the diagnostic criteria of mostly non-atherosclerotic form of the disease and also some geographical variation [35]. CAE is more predominant in males (1.7% compared to 0.2%) and in the RCA [26]. Conflicting data on the pathogenesis of CAE exists to date.
Aetiology of coronary artery ectasia

The exact aetiology of CAE is not well defined in the current literature. Despite inconsistent reports, it is attributed predominantly to atherosclerosis in 50% of cases due to common associations. In atherosclerotic ectatic coronary arteries, it is thought that CAE represents an exaggerated form of extensive vascular remodelling in response to atherosclerotic plaque formation, with extracellular enzymatic degradation playing a major role in ectatic vessel formation [36]. Furthermore, the in vivo data with intra-vascular ultrasound (IVUS) has confirmed that both arterial expansion and shrinkage can be a manifestation of aneurysmal coronary atherosclerosis, yet this study cohort was referred to as coronary aneurysm [37]. Atherosclerotic lesions within ectatic regions of the coronary arteries tend to be highly inflamed with high-risk plaques [36], and hence, high risk for clot formation documented by IVUS [38]. Diabetes Mellitus, in particular, has a negative association with the incidence of CAE [1, 39]. In contrast to earlier research, many reports have suggested that a noticeable percentage of CAE are non-atherosclerotic [1, 40].

Up to 20-30% of CAE cases are considered congenital and 10-20% are associated with inflammatory or connective tissue diseases (e.g., Ehlers-Danlos syndrome, Kawasaki disease and polycystic kidney disease) [26]. Kawasaki disease (KD) also determines the prevalence of potential coronary dilatation, affecting around 19/100,000 children in the USA [41, 42]. While in Japan the cumulative incidence of KD is high and more than 10 / 1000 of kids under 10 years of age between 1991 till 2017 are affected [43].

Studies have also reported iatrogenic coronary aneurysms after balloon angioplasty with incidence of at 0.3- 4%, with a higher incidence –up to 9%- when the angioplasty was complicated by dissection [44, 45]. Other rare causes include congenital CAD (i.e. abnormal
origin of the coronary artery), heart cavity fistula [46], syphilis, trauma or dissection. Patients with none of the above aetiologies may be described as having idiopathic CAE. In our modern clinical practice, these rare causes for CAE are almost obsolete, making an idiopathic aetiology the most widespread form of CAE [47, 48].

Takayasu Arteritis (TA) is another potential association with some cases of CAE. It is a syndrome of chronic pan-arteritis, which can be complicated by aneurysmal dilatation of the coronary arteries. It is rare to have coronary involvement with Takayasu’s arteritis, with annual incidence of 2.6 per million [49]. In a study between 1961 and 1989, out of 63 patients with TA and angina undergoing surgical intervention, coronary ostial disease was found in 73%, proximal CAD in 18%, left main ostial lesions in 67% with more than 90% occlusion or even complete occlusion [50].

Moreover, CAE was documented in various systemic conditions such as Crohn’s disease [51], rheumatic valve disease [52], varicose veins [53] and aneurysmal dilatation of the Aorta and basilar artery [54, 55]

**Morphological classification of coronary artery ectasia**

While coronary angiography is the gold standard diagnostic technique for detecting CAE, IVUS is used previously to confirm CAE morphology and assess coronary luminal size [56]. Positive remodelling may be falsely interpreted as ectasia in quantitative angiogram assessment [57]. The classic Markis classification is often used as follow [58]:

I. Type 1, diffuse ectasia of 2-3 vessels;

II. Type 2, diffuse disease in one vessel only and localised disease in another vessel;

III. Type 3, diffuse ectasia of one vessel;

IV. Type 4, localised or segmental ectasia.
It has been proposed that the term aneurysm should be restricted to a localised, abnormal dilatation of a coronary artery, which can be saccular or fusiform in shape [59], while reserving the term ectasia to describe diffuse dilatation only (>50% of the artery) or dilatation longer than 20 mm in length in other literatures [31]. Adherence to this definition criteria is needed to empower accurate estimation of CAE prevalence and hence to facilitate associations between various CAE studies.

**Pathogenesis of coronary artery ectasia**

The underlying pathogenesis of CAE is not well understood. Histologically, CAE has many similar vessel wall features to atherosclerotic CAD; hyalinization and lipid deposition in the intima, destruction of the intima and media, focal calcification, sclerosis and fibrosis [60]. The loss of the musculo-elastic arterial wall seems to be a unique characteristic of isolated CAE, which results in a marked attenuation of the vessel wall dilatation [58, 61]. The essential component in the formation of a coronary aneurysm is thought to be an abnormal arterial media, which may be secondary to an extension of the intimal arteriosclerotic process [61, 62]. Ideally, the non-atherosclerotic form of ectasia has intact intima but extensive medial degeneration where smooth muscles are replaced by hyalinised collagen. Moreover, it is becoming increasingly recognised that traditional CV atherosclerotic risk factors are not the main contributors to CAE formation. This is supported by the histological variance and a paradoxical low prevalence of CAE in diabetics [61, 63]. Furthermore, patients with solely (pure) CAE have been found to be younger, have diffuse disease involving the three main epicardial coronary arteries and have less traditional CV risk factors than those with mixed atherosclerotic CAE [63]. Hence, it appears inappropriate to label the overall CAE disease as a variant of atherosclerosis. CAE has also been found to correlate with the over-expression of matrix metalloproteinases (MMPs), which contribute to excessive vessel dilatation and
aneurysm formation. Interestingly, this is down-regulated in diabetes and may explain the lower incidence of CAE in diabetics [64].

**Inflammatory response in coronary artery ectasia**

The literature fails to provide a specific biomarker for CAE; the ones quoted are known to be already abnormal in atherosclerosis. Conventional inflammatory markers, e.g. cytokines, TNF-α and interleukin IL-6, are generally good markers for systemic inflammation, and have been found to be elevated in 50% of patients with CAE, predominantly those with evidence for atherosclerosis and infection [65]; however, they have failed to differentiate between the two conditions. Chronic inflammatory status with elevated non-specific inflammatory cells markers, e.g. leucocyte count (WCC), monocyte count, and C reactive protein (CRP), have been reported to be linked to the presence of CAE [66].

Triantafyllis et al. reported higher levels of IL-4 and lower levels of IL-2 in patients with CAE than in patients with obstructive coronary disease. Moreover, IL-6 levels were lower in individuals with normal coronary arteries than in those with obstructive coronary disease and CAE. IL6 levels were comparable between CAE and obstructive CAD. The differences in pro-inflammatory levels in this study suggested T-helper 2 cells (TH2) involvement – rather than TH1 pathway- in the immune response in CAE [67], as has also been documented by Adioglu et al. [68]. Higher levels of soluble adhesion molecules such as ICAM and VCAM were found in patients with CAE compared to those with occlusive coronary artery disease and healthy coronary vessels[69]. The CAE inflammatory response has been previously studied including common biomarkers as white cell count, CRP, adhesion molecules, soluble molecules, matrix metalloproteinases and the immune-inflammatory response (Table 1).
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Markers</th>
<th>Sub-groups in study</th>
<th>Conclusion</th>
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<tbody>
<tr>
<td>Li JJ</td>
<td>2009</td>
<td>- Cytokines</td>
<td>- 55 with isolated CAE</td>
<td>WCC, CRP and IL-6 raised in CAE compared to obstructive CAD and those with normal coronaries.</td>
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<td>- Blood cell count</td>
<td>- 38 with obstructive CAD</td>
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<td>- 33 with angiographically normal coronaries</td>
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<tr>
<td>Turhan H</td>
<td>2005</td>
<td>- Plasma soluble adhesion molecules</td>
<td>- 32 with isolated CAE without stenosis</td>
<td>Isolated CAE associated with raised ICAM-1, VCAM-1 and E-selectin.</td>
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<td>- 32 with obstructive CAD without CAE</td>
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<td>- 30 with normal coronary arteries</td>
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<tr>
<td>Dogan A</td>
<td>2008</td>
<td>- MMPs</td>
<td>- 28 with CAE</td>
<td>MMP-3, MMP-9 and IL-6 may be responsible for ectasia. hsCRP similar in all three groups.</td>
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<td></td>
<td></td>
<td>- ILs</td>
<td>- 27 with CAD</td>
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<td></td>
<td></td>
<td>- Inflammatory markers</td>
<td>- 22 with angiographically normal coronaries</td>
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<tr>
<td>Daoud EM</td>
<td>2012</td>
<td>- Plasma soluble adhesion molecules</td>
<td>- 16 with isolated CAE</td>
<td>ICAM-1 significantly higher is isolated CAE. E-selectin was not different between groups</td>
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<td></td>
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<td>- 16 with obstructive CAD</td>
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<td>- 10 with angiographically normal coronaries</td>
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<tr>
<td>Adiloglu AK</td>
<td>2005</td>
<td>- ILs</td>
<td>- 88 with three or more obstructed vessels</td>
<td>HsCRP and IL-6 were higher in CAE compared to controls.</td>
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<td>- Inflammatory markers</td>
<td>- 65 with CAE without atherosclerosis</td>
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<td></td>
<td>- 91 with angiographically normal vessels</td>
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<td>Yilmaz H</td>
<td>2006</td>
<td>- Adhesion molecules</td>
<td>- isolated CAE without CAD</td>
<td>Significantly increased ICAM-1 and VCAM-1 in patients with CAE and those with obstructive CAD with CAE.</td>
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<td>- obstructive CAD and CAE</td>
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<td>- normal coronary arteries</td>
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<tr>
<td>Turhan H</td>
<td>2004</td>
<td>- Inflammatory markers</td>
<td>- 32 with isolated CAE</td>
<td>CRP significantly higher in those with isolated CAE</td>
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<td>- 32 with CAD without CAE</td>
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<td>- 30 with angiographically normal coronaries</td>
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<td>Finkelstein A</td>
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<td>- 34 with isolated CAE</td>
<td>Serum levels of MMP-2, MMP-3, TIMP-1, proBNP and hsCRP did not differ between the three groups.</td>
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<td>- Inflammatory markers</td>
<td>- 26 with CAD without CAE</td>
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<td>- 27 with angiographically normal coronaries</td>
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Metabolic profiling of coronary artery disease and coronary ectasia

Metabolites detailed analysis is a relatively new technology in the medical field that focuses on the comprehensive assessment of endogenous metabolites processing and attempts to systematically identify and quantify metabolites pathways from a biological origin. Metabolites represent a diverse group of low-molecular-weight structures including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols and carbohydrates, which make global analysis a difficult challenge. The recent rapid development of analytical platforms enables the process of separation, detection and quantification of metabolites and their patterns of presentation. Metabolomics analysis has been shown to promise immense potential for early diagnosis [75], therapy monitoring and for understanding the pathogenesis of many diseases in the area of cancer, diabetes, inborn errors of metabolism and CV diseases [76-78]. To identify, in modern practice, the putative biomarkers that serve as a valuable early diagnostic tool is quite challenging. However, these metabolite markers would identify a subset of patients with increased risk for developing certain diseases. This offers a comprehensive disease prediction tool and could improve diagnostic precision [79]. Since CAE has a unique and not well-understood pathogenesis, that is distinct from the well-defined atherosclerosis process, lipid metabolites profiling and analysis are likely to lead to a better understanding of its pathophysiology, which could argue against the common concept of CAE involvement with atherosclerosis [80].

Clinical and pathological evaluations of coronary artery ectasia

Coronary artery ectasia is considered as an incidental finding in most clinical settings. Most clinicians would manage CAE similar to non-dilated coronaries in the acute stages. However, CAE has a different pathophysiology that carries potential different outcome. We hereby highlight the main considerable differences between the two pathologies:
A- Prognosis of coronary artery ectasia in acute coronary syndrome

The managements’ recommendations for patients presenting with ST elevated myocardial infarction (STEMI) with an ectatic infarct-related artery (EIFA) are the same as for a STEMI in the absence of CAE. The main target is to achieve early revascularization. [81]. Nevertheless, A recent study found that patients presenting with STEMI due to an EIFA were more likely to have a large thrombus burden (96% vs 22%), increased additional usage of glycoprotein IIb / IIIa inhibitors (GPI) (73% vs 37%), and greater post-procedural anticoagulation use (28% vs 5%). Increased bleeding rates were not observed despite the increased use of GPI [82]. Patients with concomitant CAD and CAE have higher no-reflow rates and lower TIMI flow grades after percutaneous intervention [56, 82, 83]. Overall, despite high-burden thrombus formation and lower rates of successful reperfusion, long term survival tends to be good [7]. The presence of CAE in acute coronary syndrome (ACS) makes stenting more arduous. Challenges include optimal stent sizing, stent misplacement, stent embolization and acute or sub-acute stent thrombosis [84]. In one study, 44% of patients presenting with STEMI and concomitant CAE did not receive a stent. The authors of this study attributed these complications to the large vessel size, persistence of the large thrombus burden even after thrombus aspiration and the initiation of GPI [82]. Additional low rates of stenting have been noted in another study, however, the use of PCI/Stents revealed better in hospital outcome post cardiac events [85]. There are no clear recommendations on the optimal strategy for revascularization (i.e. clot aspiration, GPI use, stent or bypass surgery) in CAE with ACS. A randomised controlled trial has assessed the use of Everolimus-eluting, Sirolimus-eluting and bare metal stents in patients requiring stents of 3.0 mm or more in diameter to match dilated segment diameter in CAE. These reported lower rates of future target revascularisation with the drug eluting stents. Moreover, no significant differences in
the long-term rates of mortalities and MI recurrences in those receiving drug-eluting stents compared to bare metal stents[86].

B- Coronary slow flow
The presence of dilated/ectatic segments produces sluggish and turbulent blood flow. This is associated with an increased incidence of typical exercise-induced angina pectoris and MI, regardless of the severity of the co-existing coronary stenosis [57]. This phenomenon is known as coronary slow flow (CSF) and is characterised by protracted distal vessel opacification in the absence of significant stenotic epicardial CAD. The TIMI frame count method (TFC), which is an index of coronary flow velocity along the entire coronary artery, can also be used to assess CSF. Coronary ectasia is associated with an increased TFC [87]. It has been suggested that while volumetric coronary blood flow is significantly higher in CAE, the average peak velocities of coronary blood flow during hyperaemia is significantly lower than that in normal coronary arteries [88]. The coronary flow reserve also appears to be reduced in patients with CAE. This has led to the suggestion that microcirculatory dysfunction may cause exercise-induced ischaemia [88].

C- Coronary artery ectasia related arrhythmia
Few studies have analysed the electrocardiogram (ECG) changes in patients with CAE. One small study (n=20) found that isolated CAE was associated with a prolonged dispersion of the P-wave and QT interval [89]. P wave dispersion (i.e. difference between longest and shortest P wave durations in the 12 leads ECG in millisecond) was higher in isolated CAE patients. This correlated with higher incidence of electrical dysfunction and remodelling hence increasing the dyssynchrony of inter atrial contractions and consequently the elevated occurrence rate of atrial fibrillation [90-94].
Dispersion of QTc represents the difference between the maximum and minimum QTc durations on 12 leads ECG. It is emerging as an important clinical tool to predict the development of ventricular arrhythmias especially in patients with a history of CAD [95, 96]. Despite the few available evidence about prolonged QTc dispersion in CAE, however, theoretically it can be secondary to slow flow and induced micro ischemic culprits [97, 98].

A more recent study assessed the Tp-Te (the interval between the peak and end of the T-Wave) and Tp-Te/QT interval in patients with CAE [96]. Tp-Te is accepted as an index of transmural dispersion of ventricular repolarisation. Myocardial repolarisation abnormalities are associated with susceptibility to ventricular tachy-arrhythmias, hence the use of Tp-Te interval and Tp-Te/QT ratios as electrocardiographic indexes of ventricular arrhythmogenesis [99],[100, 101]. Patients with CAE had significantly higher values of Tp-Te and Tp-Te/QT than those with normal coronary arteries. Authors concluded that patients with CAE may carry an increased arrhythmia risk [96]. An increased Tp-Te interval and Tp-Te/QT ratio has also been found in those with isolated CSF, a phenomenon which is often associated with CAE [102]. Further studies are required to examine if these findings extrapolate to larger populations with CAE.

**Management of coronary artery ectasia**

Management of Coronary artery ectasia falls mostly under the umbrella of clinical experience and the diagnosis of the disease remains accidental especially for isolated form of the disease without previous history during childhood and adolescence. The diagnostics and treatment options are enumerated here.
I- Diagnostic Imaging for coronary artery ectasia

I- A) Cardiovascular magnetic resonance (MR): Cardiovascular MR has been successfully used for simultaneous evaluation of myocardial function and the presence of myocardial inflammation but remains limited in its accurate assessment of the coronary arteries because of its poor temporal resolution [103, 104].

I- B) CT coronary angiography: Multi–detector CT angiography provides accurate description of vessel origin and course, particularly in anomalous vessels [40]. It is a viable noninvasive imaging modality for defining coronary arterial anomalies, particularly if findings at coronary angiography are not conclusive [105]. In a cohort of 577 patients, CT coronary angiography identified 1.7% with CAE [106].

I- C) Echocardiography: Echocardiography is of specific value for diagnosing ectatic coronary arteries related to Kawasaki Disease especially in demonstrating proximal coronary ectasia anatomy and through assessing ventricular size and function by performing stress-shortening and stress-velocity analysis [107]. The value of this technique in assessing proximal coronary arteries in adults is no more than modest, particularly with the advent of other direct imaging modalities e.g. CT Stress echocardiography is of great value in assessing the effect of coronary lesions on myocardial function, irrespective of patient’s age. However, from all the above modalities, it appears that CT coronary angiogram remains the most practical test after the gold standard invasive coronary angiography[108].

II- Treatment of coronary ectasia:

a) Anticoagulation and antiplatelet indications in CAE management

Guidelines on the appropriate treatment for CAE do not exist. Evidence to date is from case reports and studies limited by population size (Table 2). Older studies recommended the use
of long-term anti-coagulation based on significant flow disturbances within the ectatic segments; however, this is controversial [109, 110]. The European Society of Cardiology notes that chronic oral anticoagulation has not been prospectively verified and hence cannot be recommended until supported by subsequent studies. Anti-platelet agents are administered to the majority of patients, as there is a high prevalence of co-existing obstructive CAD. The increased platelet activation in isolated CAE (unregulated P-selectin, beta-thromboglobulin and platelet factor IV) further supports the use of anti-platelet agents in CAE [111]. In contrast to obstructive CAD, the use of nitrates is generally discouraged. Nitrates may cause further coronary epicardial dilation, which can exacerbate myocardial ischaemia (i.e. excessive intimal wall hyalinization and slow coronary flow) [112, 113]. Statins may have an additional role in CAE as they can inhibit the secretion of MMPs enzymes and supress inflammatory cascades [114].

b) Surgical vs. PCI treatment options in coronary artery ectasia

Percutaneous or surgical intervention is often used when symptoms fail to respond to medical treatment. A small study conducted in 1990 found that patients undergoing angioplasty for lesions adjacent to an aneurysmal coronary artery segment had similar outcomes to those with obstructive CAD without CAE [115]. Patients who present with STEMI, due to an ectatic infarct–related artery (EIRA), have a better in-hospital prognosis when percutaneous coronary intervention (PCI) is performed [82]. A number of surgical procedures have been used to treat CAE with or without co-existing obstructive CAD. While the most common procedure is a coronary artery bypass graft (CABG), proximal and distal ligation and aneurysm resection have been used to remove large aneurysms with high burden of thrombus formation within this CAE segment [116]. This paper has presented an experience in few cases without clear definition and separation of ectasia from aneurysm. Recent studies
confirmed that surgical treatment of large coronary aneurysms in 15 patients had good short-and long-term results [117].

Table 2. Anticoagulation and revascularisation treatment options in coronary artery ectasia

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study details</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swanton RH [109]</td>
<td>1978</td>
<td>1000 angiograms 12 had CAE</td>
<td>Anticoagulation recommended</td>
</tr>
<tr>
<td>Demopoulous VP</td>
<td>1997</td>
<td>Group A - 172 with CAE and CAD</td>
<td>No additional risk in CAD with concurrent CAE.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group B - 31 with isolated CAE</td>
<td>Use of anticoagulants should be questioned.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group C - 165 with CAD without CAE</td>
<td></td>
</tr>
<tr>
<td>Sorrell VL [118]</td>
<td>1998</td>
<td>Review</td>
<td>Warfarin, aspirin and diltiazem recommended</td>
</tr>
<tr>
<td>Shanmugam BV [82]</td>
<td>2017</td>
<td>1834 primary PCI patients - 25 had EIRA - 1809 had non-EIRA</td>
<td>Improved in-hospital outcomes with PCI High frequency of unstable angina and non-fatal MIs in both groups after discharge</td>
</tr>
</tbody>
</table>

CAE: coronary artery ectasia, PCI: percutaneous coronary intervention, EIRA: ectatic infarct related artery, MI: myocardial infarction

III- Cardiovascular events in coronary ectasia after myocardial infarction

The clinical progression reports and long-term outcomes of Coronary artery ectasia - after acute presentations- are limited. In some studies, the isolated CAE seems to have a similar prognosis to that of obstructive CAD [35, 119, 120]. One of the largest studies compared the short term outcome over a 30 month period between 92 patients with CAE and 114 patients with significant CAD without ectasia [35]. This study indicated higher incidences of unstable angina presentations in patients with isolated CAE, although the incidence of myocardial infarction and cardiac death did not differ significantly between the two groups. The same
study found the incidence of PCI was less required in isolated CAE, whereas the incidence of CABG was marginally higher compared to those with significant CAD without ectasia [35]. However, it appears that patients who have a STEMI due to an Ectatic infarct-related artery (EIRA), may have a worse long-term outcome than those without EIRA [82]. Additional factor attributed to a worse prognosis is a high thrombus burden in CAE, which can lead to poor reflow after revascularizations intervention [7].
**Dissertation objectives**

This thesis aims to identify some of the clinical features of CAE and to unravel part of its pathogenesis. The thesis has the following four objectives:

**Paper One:**
To assess the short-term outcome of CAE in acute MI presentations in comparison to atherosclerotic CAD outcome. It also compares the standard inflammatory responses, that associating acute MI in the two conditions.

**Paper Two:**
To investigate the personalized metabolic profile of CAE, by explaining the lipid and fatty acid metabolite lineage that may help to shed the light on some aspects of the pathophysiology of CAE.

**Paper Three:**
To study the immune-inflammatory response associated with CAE by analysing cytokine response of CAE in detail and by identifying the pathophysiological inflammatory pathways in patients with CAD and normal coronary arteries.

**Paper Four:**
To evaluate the long-term follow up outcome and prognosis of CAE patients. The study is to analyse all hospital readmissions with MACE and CV mortalities.
Subjects and Methods

Coronary ectasia was identified as a dilated segment more than 1.5 times the diameter of the neighbouring artery and involving more than 1/3 of the artery length. Pure CAE was characterised as smooth coronary arteries with no evidence of atherosclerosis. Mixed CAE was characterised as having minimal evidence of atherosclerotic changes (plaque formation or minor atheromata ≤ 20% of lumen narrowing or causing some lumen irregularities). No structural heart disease.

Paper One

Retrospective examination was carried out on 3,321 consecutive acute MI patients who underwent primary PCI in two centres in the United Kingdom (Royal Free Hospital, London and Norfolk, and Norwich University Hospital) between January 2009 and August 2012. We identified 30 patients (mean age 54.7 ± 15.1 years, 5 female) with CAE and compared them with an age- and gender-matched control group of 60 atherosclerotic CAD having acute MI. CAE was defined via the conventional definition as a dilatation more than 1.5 times the diameter of adjacent artery and also more than 20 mm in length [31]. The CV risk factors were assessed from patient’s medical records. Clinically available acute inflammatory markers, including full blood counts and CRP were also documented. Major acute cardiac events “MACE” and mortality were documented over a short-term follow up period of 2 years.

Statistical analysis

Statistical Package of Social Science version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables were expressed as mean SD and categorical
variables as absolute value and percentage. We compared means of independent samples using the unpaired Student t test or Mann–Whitney as appropriate and proportions using the Chi-square test. Kaplan–Meier curve was used to represent the short-term MACE outcomes.

**Paper Two**

Serum samples from 16 patients with CAE (mean age 63.5 ± 10.1 years, 6 female) were compared to 26 controls with normal (smooth) coronary artery (mean age 59.2 ± 6.6 years, 7 female). Plasma extractions and preparations were performed according to the Swedish Metabolomics Centre protocol, Umeå, Sweden. Light chromatography-mass spectrometry (LC-MS) analysis and metabolite identification were performed at the second stage. Mass feature extraction (MFE) from the data acquired was performed using Qualitative Analysis and then matched using mass profile identification software of metabolites library. Principal components analysis (PCA) is an unsupervised multivariate method that was used for data overview and to detect trends and outlier. Projection to latent structure discriminant analysis (PLS-DA) a variant of the previous PLS methods was used when Y is categorical, as when discriminating between different classes.

**Statistical Analysis:**

Univariate t-tests at the 95% confidence level considering equal variances were performed, as well as Mann-Whitney U-tests. Benjamini-Hochberg correction with $\alpha=0.05$ was used during t-tests and Mann-Whitney U-test to control the False Discovery Rate. All calculations performed in MATLAB® (MathWorks®, Natick, USA).

Principal Components Analysis (PCA) is an unsupervised multivariate method used for data overview and to detect trends and outliers. The principal components represent the directions of largest variance in the data, approximating the data as well as possible in the least squares
sense. It allows simpler visualization by reducing data dimensionality and separating information from random variation.

Projection to Latent Structures (PLS) is a (supervised) multivariate regression method related to PCA. Orthogonal Partial Least Squares (OPLS), a modification of the PLS method, finds the relations between two matrices (data X and response Y), by maximizing the covariance of their latent variables. It allows understanding which variables are more correlated to a response and making predictions for the new samples. It provides better interpretation of the relevant variables than PLS. It does so by decomposing the data in so-called “predictive” information related to the response Y (as concentrations, classes), “orthogonal” structured information not related to the response (as instrumental, biological variations), and residual variation.

(O)PLS-DA (Discriminant Analysis) is a variant of the previous (O)PLS methods, used when Y is categorical, as when discriminating between different classes [121].

Statistical significance of metabolites and features was considered if the OPLS-DA loadings p was larger than its confidence interval (the confidence interval had not cross zero), and simultaneously the t-test and Mann-Whitney U-test had p-values lower than 0.05 after Benjamini-Hochberg correction.

**Identification of statistically significant signals detected by UPLC- MS:**

Mass Feature Extraction (MFE) - of the metabolites- from the data acquired was performed using the MassHunter™ Qualitative Analysis software package, version B06.00 (Agilent Technologies Inc., Santa Clara, CA, USA). Extracted features were aligned and matched between samples using Mass Profiler Professional™ 12.5 (Agilent Technologies Inc., Santa
Clara, CA, USA). In-house database with exact mass and experimental retention times of lipids were used for identification.

**Paper Three**

Two hundred and twenty-five serum samples (from CAE patients, CAD and controls) were assayed in duplicate using the MSD pro-inflammatory Panel I, a highly sensitive multiplex enzyme-linked immunosorbent assay (ELISA). This was used to quantitatively measure the levels of 10 cytokines including IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNFα. The lowest detection limit (LLOD) was calculated according to the manufacturers’ protocol and the mean value from three plates was used for further calculation of the sample concentrations.

Patients with CAE were selected from 16,464 angiograms performed between 2003-2011 in Umeå university Heart Centre, Sweden and Letterkenny university hospital, Ireland. The same inclusion criteria were applied (detailed in paper 2) and then 66 patients were identified where 16 responded to the invitation for blood samples recruitment and project participation.

We also studied another group of 69 patients with evidence of mild non-obstructive coronary artery disease and a third group of 140 controls, whose angiogram showed completely normal coronary arteries.

**Statistical analysis**

All quantities of samples of coronary ectasia and controls were listed. Differences between patients and controls were assessed using Mann-Whitney U-tests. The data was reported in a descriptive method of median and interquartile values. Statistical significance was defined as p<0.05. Points were shown as outliers if they were larger than Q3+2*IQR or smaller than Q1-2*IQR where Q1 and Q3 are the 25th and 75th percentiles, and IQR=Q3-Q1.
Analyses were performed with IBM SPSS Statistics program for Macintosh Version 24.0 (IBM Corp, Armonk, NY).

**Paper Four:**
A cohort of 66 patients with CAE, diagnosed among 16,464 patients who underwent diagnostic coronary angiograms in Umeå Heart Centre, Sweden and Letterkenny university Hospital, Ireland between 2003 and 2009. Long-term follow up (median 10, IQR “9.7 – 10.3” years) data on Major Acute Cardiac Events (MACE, i.e.; readmission with ACS, MI, and death from the cardiac events) were collected from 41 patients (age median 61 IQR “56 – 68” years, 12 Female). We recruited a control group of 41 patients, who were selected consecutively with minimal coronary artery disease (CAD) (≤ 20% luminal narrowing on conventional coronary angiography). Follow up period included all patients who underwent coronary angiogram between the period from 2003 and 2011. Follow up period till December 2018. (Figure 1)

We excluded patients or controls that had prior coronary intervention, more than mild valve disease, or congenital heart disease at the time of the diagnostic coronary angiogram. The study was approved by the Regional Ethics Committee of Umeå (Sweden) and Letterkenny University Hospital (North West Health Service Executive, Ireland).

**Statistical analysis:**
Statistical analyses were performed using IBM SPSS Statistics program for Macintosh Version 24.0 (IBM Corp, Armonk, NY). The data were reported as median (IQR), or as number and per cent of subjects. Differences between patients and controls were assessed using Mann-Whitney U-tests. Proportions were analysed by chi square tests or Fisher’s exact test, as appropriate. Since our hypothesis was that the number of deaths/MACE was higher in
the CAE group/non-survivors, one-sided tests Fisher’s exact tests were utilized. However, two-sided tests were used to compare the prevalence of risk factors in different groups, since we expected that the prevalence could be both higher and lower in the group with the highest risk of cardiac events. Statistical significance was defined as p<0.05

Figure 1: This figure summarizes patients’ selection and follows up data of CAE patients.
Results

Paper One

*Coronary ectasia prevalence in ACS, demographics and cardiovascular risk profile*

The prevalence of CAE among the whole cohort of patients presenting with acute MI was 2.7%. The conventional risk factors including hypertension, hypercholesterolemia, family history of CAD and history of smoking were not significantly different between CAE and non-CAE patients. However, diabetes mellitus (DM) was less common in the CAE group (p=0.02) (Table 3).

**Table 3. Cardiovascular and demographic comparison between coronary artery ectasia (CAE) and patients without CAE**

<table>
<thead>
<tr>
<th></th>
<th>CAE N=30</th>
<th>Non-CAE N= 60</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis</td>
<td>9 (30%)</td>
<td>56 (93.5%)</td>
<td>0.01*</td>
</tr>
<tr>
<td>RCA</td>
<td>23 (76.7%)</td>
<td>21 (35 %)</td>
<td>0.01*</td>
</tr>
<tr>
<td>LAD</td>
<td>14 (46.7%)</td>
<td>29 (48 %)</td>
<td>0.82</td>
</tr>
<tr>
<td>LCX</td>
<td>15 (50%)</td>
<td>4 (6 %)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (43.3%)</td>
<td>22 (36 %)</td>
<td>0.82</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>17 (56.7%)</td>
<td>28 (46 %)</td>
<td>0.65</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>3 (10%)</td>
<td>19 (31 %)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Smoking history</td>
<td>24 (80%)</td>
<td>34 (56 %)</td>
<td>0.43</td>
</tr>
<tr>
<td>FHx of CAD</td>
<td>15 (50%)</td>
<td>22 (36 %)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

LAD: Left anterior descending artery, RCA: Right coronary artery, LCX: Left circumflex artery, CAD: Coronary artery disease, FHx: Family history
Anatomical description of the culprit coronaries in coronary ectasia

Myocardial infarctions (MIs) related to high-grade atherosclerosis stenosis were less documented in CAE group (9 (30%) vs 56 (93.3%), p=0.001). The RCA and LCx were predominantly involved in patients with CAE (p=0.001 and p=0.0001, respectively). The LAD was the culprit in most patients presented with acute MI, irrespective of the presence of CAE (p = 0.82). Culprit lesions were identified, angiographically or via ECG changes (Q wave/ST changes), (7) in 20 out of 30 (66.6%) patients with CAE. However, 14 (46.7%) patients with CAE had diffuse involvement (>2 lesions in two or more of coronaries).

Myocardial infarction-related inflammatory markers in coronary artery ectasia

Although CRP was higher (p=0.006) in patients with CAE, yet WCC, neutrophil and neutrophil/lymphocyte ratio (N/L ratio) were lower in patients with CAE (p=0.002, 0.002 and 0.032, respectively). The platelets count did not differ with CAE (Table 4). The leucocyte, neutrophil response and N/L ratio were higher in patients with a single and localized CAE culprit compared to those with diffuse CAE (p=0.02, 0.008 and 0.03, respectively) (Table 4 and Figure 2).
Table 4. Comparison of inflammatory response between coronary artery ectasia (CAE) and patients without CAE; sub-analysis of diffuse vs localised CAE lesions

<table>
<thead>
<tr>
<th></th>
<th>CAE (N=30)</th>
<th>Non-CAE (N=60)</th>
<th>P value</th>
<th>Localised CAE (N= 16)</th>
<th>Diffuse CAE (N= 14)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>57.1±12.2</td>
<td>55.8±11.3</td>
<td>0.76</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>36.7± 49.7</td>
<td>8.9± 16.1</td>
<td>0.006*</td>
<td>27.5±26.8</td>
<td>47.9±67.9</td>
<td>0.28</td>
</tr>
<tr>
<td>Platelets (x10^9/L)</td>
<td>243± 66</td>
<td>247± 92</td>
<td>0.84</td>
<td>258±72</td>
<td>226±56</td>
<td>0.19</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>1.84± 0.60</td>
<td>1.99±0.95</td>
<td>0.49</td>
<td>1.90±0.51</td>
<td>1.75±0.72</td>
<td>0.51</td>
</tr>
<tr>
<td>WCC (x10^9/L)</td>
<td>9.3± 4.3</td>
<td>12.2± 3.9</td>
<td>0.002*</td>
<td>10.87±4.84</td>
<td>7.31±2.63</td>
<td>0.02*</td>
</tr>
<tr>
<td>Neutrophils (x10^9/L)</td>
<td>6.55±0.36</td>
<td>9.10±3.31</td>
<td>0.002*</td>
<td>8.86±4.80</td>
<td>4.26±1.63</td>
<td>0.01*</td>
</tr>
<tr>
<td>N/L ratio</td>
<td>3.9± 2.9</td>
<td>6.0± 4.7</td>
<td>0.032*</td>
<td>4.97±3.47</td>
<td>2.59±1.22</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

* Statistically significant. N/L ratio: Neutrophil/lymphocyte ratio, WCC: White cells count

Figure 2. White cell count (WCC), neutrophil and C-reactive protein (CRP) levels are significantly different between coronary artery ectasia (CAE) and atherosclerotic coronary artery disease (CAD) patients.
Inflammatory response of totally occluded coronary artery disease and ectasia

The WCC and neutrophil count were higher in patients with total occlusion of CAE and with heavy thrombus burden (defined angiographically as total occlusion with no coronary flow) compared to those with only significant stenosis (both p=0.02) in (Table 5). Patients with CAD with total occlusion had also higher WCC and neutrophil level (p=0.004 and p=0.003, respectively) than those with non-obstructive stenotic lesions. Despite of CRP higher trends in both groups of CAE and CAD, but no statistical differences were observed (Table 5).

Table 5. Inflammatory response based on the type of the angiographic coronary lesion; total occlusion vs tight stenosis in the entire cohort and in coronary artery ectasia patients only

<table>
<thead>
<tr>
<th></th>
<th>CAD Stenosis</th>
<th>CAD Occlusion</th>
<th>P value</th>
<th>CAE Stenosis</th>
<th>CAE Occlusion</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x10^9/L)</td>
<td>250.3±63.0</td>
<td>243.1±57.5</td>
<td>0.65</td>
<td>247.0±67.4</td>
<td>239.8±66.5</td>
<td>0.77</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>13.7±24.1</td>
<td>32.6±51.5</td>
<td>0.10</td>
<td>19.1±30.5</td>
<td>53.0±59.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>1.88±0.74</td>
<td>1.92±0.71</td>
<td>0.86</td>
<td>1.91±0.76</td>
<td>1.81±0.44</td>
<td>0.64</td>
</tr>
<tr>
<td>WCC (x10^9/L)</td>
<td>7.87±2.86</td>
<td>11.78±4.46</td>
<td>0.004</td>
<td>7.42±2.08</td>
<td>11.0±5.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Neutrophils (x10^9/L)</td>
<td>5.88±2.71</td>
<td>8.64±4.28</td>
<td>0.003</td>
<td>4.63±1.72</td>
<td>8.19±5.02</td>
<td>0.02</td>
</tr>
<tr>
<td>N/L ratio</td>
<td>3.75±2.38</td>
<td>5.41±3.89</td>
<td>0.07</td>
<td>2.86±1.80</td>
<td>4.88±3.46</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* N/L ratio: Neutrophil/lymphocyte ratio   *CAD: coronary artery disease   *CAE: Coronary artery ectasia

Short-term follow up and major acute cardiac events

The short-term follow-up period of 2 years revealed no relationship between the inflammatory markers levels and MACE (8/28, 28.6%) in patients with CAE vs (13/60, 21.7%) in those without CAE; (p=0.42). Two patients were lost during follow-up period.
The Kaplan-Meier curve showed no significant difference in MACE rate between CAE and patients without CAE (p=0.08) (Figure 3). Similarly, there was no significant difference in the cardiovascular mortality over the 2 years’ follow-up between both groups (p=0.15).

**Figure 3.** Kaplan-Meier curve showing no difference in major acute cardiac events (MACE) between patients with and without CAE over the 2-year follow-up period.

**Relationship between inflammatory response and treatment options**

Inflammatory markers serum values failed to predict and differentiate between the management options of medical treatment, PCI or CABG (Table 6).
Table 6. Management options of CAE/ACS based on inflammatory markers response

<table>
<thead>
<tr>
<th></th>
<th>Medical</th>
<th>PCI ± STENT</th>
<th>CABG</th>
<th>P (one-way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>243±67</td>
<td>235±69</td>
<td>280±65</td>
<td>0.70</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>39.1±60.6</td>
<td>32.1±28.5</td>
<td>35.0±9.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Lymphocytes (x10^9/L)</td>
<td>1.82±0.67</td>
<td>1.97±0.53</td>
<td>1.69±0.30</td>
<td>0.78</td>
</tr>
<tr>
<td>WCC (x10^9/L)</td>
<td>7.99±3.26</td>
<td>11.10±5.02</td>
<td>12.67±7.87</td>
<td>0.11</td>
</tr>
<tr>
<td>Neutrophils (x10^9/L)</td>
<td>5.13±2.92</td>
<td>8.31±4.83</td>
<td>10.29±7.76</td>
<td>0.06</td>
</tr>
<tr>
<td>N/L ratio</td>
<td>3.30±2.56</td>
<td>4.51±2.90</td>
<td>6.62±6.60</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*CABG: Coronary artery bypass graft, **PCI: Percutaneous catheter intervention

Paper Two

Demographics and cardiovascular risk factors

Patients with CAE and control group demographics are depicted in (Table 7). All CV risk factors were not significant between the two groups.

Table 7. Demographics and cardiovascular risk factors in coronary artery ectasia (CAE) patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Ectasia group (N= 16)</th>
<th>Control (N= 26)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (Female, %)</td>
<td>(6, 38%)</td>
<td>(7, 27 %)</td>
<td>N/A</td>
</tr>
<tr>
<td>Age (mean ± SD) years</td>
<td>63.5 ± 10.1</td>
<td>59.2 ± 6.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (N, %)</td>
<td>10, 62 %</td>
<td>17, 65 %</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes Mellitus (N, %)</td>
<td>(4, 25 %)</td>
<td>(4, 15 %)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidaemia (N, %)</td>
<td>9, 56 %</td>
<td>16, 54 %</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>25.8 ± 4.5</td>
<td>27.1 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of IHD (N, %)</td>
<td>7, 38 %</td>
<td>(12, 46 %)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (N, %)</td>
<td>7, 43 %</td>
<td>12, 46 %</td>
<td>NS</td>
</tr>
</tbody>
</table>
Identified metabolites in coronary artery ectasia patients vs controls

We separated 65 metabolites differed between the two groups; however, only 27 metabolites were identified using the Mass feature extraction (MFE) software (i.e. 15 were fully identified, while 12 were identified through the size of the side chains). The main identified metabolites were 16 phosphatidylcholines (PC); these were dysregulated with a uniform pattern of lower concentrations in the CAE group. Likewise, the 11 sphingomyelins (SM) species had lower intensities in patients with CAE (Figure 4).

Figure 4. Boxplots for each recognised metabolite in coronary artery ectasia (CAE) patients “red” and controls “blue”. All the compared metabolites are significantly different in quantity in both groups; validation tests have confirmed this significance.
Metabolites identification methods in coronary artery ectasia

We enumerated metabolite names (and side chain sizes) that were statistically different in their concentrations between the two groups. We also reported the adduct molecular formula, retention time and the detected ionic mass in Table 8. Fold change was calculated as 

\[
\frac{\text{median}_{\text{CAE}} - \text{median}_{\text{control}}}{\text{median}_{\text{control}}}, \quad \text{with “p-value BH” is that of the 95% CI of the t-test after Benjamini-Hochberg multiple test correction (p=0.007–0.043). Additionally, it was observed that all p-loadings in the PLS-DA model were in agreement with the fold changes.}
\]

Table 8. Metabolite names (and side chain sizes) that discriminate the identified metabolites between CAE vs. controls, together with the adducts molecular formula, retention time, observed ionic mass found, fold change and BH- p value
Metabolites separation and validation

PLS-DA models of CAE vs controls showed statistical significance for both positive and negative modes separately. Validation of the models was performed using the predictive performance measure Q2, cross-validated scores (CV-scores), cross-validation analysis of variance (CV-ANOVA) and a permutation test (Table 9).

Table 9. Statistics obtained for the models of CAE vs controls in positive and negative modes, indicating the number of PLS-DA components, variance of X and Y explained by the model (R2X, R2Y), variance of Y predicted by the model (Q2), CV-ANOVA p-value and permutation test p-value.

<table>
<thead>
<tr>
<th>Group Comparison</th>
<th>CAE Vs. Control</th>
<th>CAE Vs. Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO. of components</td>
<td>Negative 1+0</td>
<td>Positive 1+0</td>
</tr>
<tr>
<td>R2X (cum)</td>
<td>0.247</td>
<td>0.163</td>
</tr>
<tr>
<td>R2X (cum)</td>
<td>0.379</td>
<td>0.388</td>
</tr>
<tr>
<td>Q2Y (cum)</td>
<td>0.172</td>
<td>0.180</td>
</tr>
<tr>
<td>CV-ANOVA (p-value)</td>
<td>0.027</td>
<td>0.030</td>
</tr>
<tr>
<td>Permutation testing (p – value)</td>
<td>0.006</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Examining scores and cross-validated scores, showed that most samples could be modelled and predicted. The ones that could not be adequately modelled and predicted present very similar behaviour in both modes. Thus, positive and negative mode datasets seemed to be in fair agreement, as both were able to model a similar class difference (Figure 5).
Figure 5. CAE vs control models for both positive (top left: scores; bottom left: CV-scores) and negative (top right: scores; bottom right: CV-scores) modes. There is major agreement between the two sets.

Pure coronary ectasia vs. mixed

To differentiate and identify metabolites, we examined the OPLS-DA models in 6 patients with pure CAE and compared with 10 patients with mixed CAE. However, the positive mode OPLS-DA model did not recognise any identifiable metabolites with significant differences in intensities between the subgroups. And although, in the negative mode, the validation methods were not clearly statistically valid with a cross-validation Q2 of 0.398, a CV-ANOVA p-value of 0.039, however, and a permutation test p-value of 0.02. The cross-validated scores can be observed in (Figure 6).
Due to multiple testing corrections and the limited number of samples, we highlighted only the negative model that identified significant metabolites. Nonetheless, the following metabolites had a valid OPLS-DA with a non-corrected p-value <0.05: SM (d18: 1/16:0), p=0.026; phosphocoline (d18: 2/17:0), p=0.0003 and SM (d18: 1/24:2), p=0.0196 had elevated concentrations in pure CAE (Figure 7). Thirteen other non-identified metabolites (10 SM and 3 PC) had a trend to be higher in pure than in mixed CAE.

Figure 6. Cross validation for (left) scores and (right) CV-scores of the OPLS-DA model of pure vs mixed CAE obtained using the data from LC-MS in the negative mode.

Figure 7. The most significant three metabolites identified with p < 0.05 in pure and mixed CAE, and controls.
Paper Three

Demographics and cardiovascular risk factors

Cardiovascular risk factors and patient baseline demographics are presented in (Table 10). There was no significant difference between CAE and CAD groups in relation to age, gender, hypertension, hyperlipidaemia, DM, family history of ischemic heart disease and smoking status. However, control group were slightly younger than CAE and include fewer subjects with hypertension and hyperlipidaemia.

Table 10. Demographic characteristics and cardiovascular risk factors in the coronary artery ectasia (CAE), CAD and controls

<table>
<thead>
<tr>
<th></th>
<th>CAE Patients (n = 16)</th>
<th>Controls (n = 140)</th>
<th>p-Value CAE vs. Control</th>
<th>CAD Patients (n = 69)</th>
<th>p-Value CAE vs. CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Female, %)</td>
<td>6 (38%)</td>
<td>81 (58%)</td>
<td>NS</td>
<td>41 (59%)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (mean ± SD) years</td>
<td>64.9 ± 7.3</td>
<td>58.6 ± 4.1</td>
<td>0.011</td>
<td>64.5 ± 8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (N, %)</td>
<td>9 (56.2%)</td>
<td>34 (25%)</td>
<td>0.01</td>
<td>49 (71%)</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes Mellitus (N, %)</td>
<td>4 (25%)</td>
<td>22 (15.7%)</td>
<td>NS</td>
<td>11 (15.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidaemia (N, %)</td>
<td>9 (56%)</td>
<td>18 (25%)</td>
<td>0.001</td>
<td>51 (74%)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (mean ± SD)</td>
<td>25.8 ± 4.5</td>
<td>27 ± 4.4</td>
<td>NS</td>
<td>27.2 ± 5.6</td>
<td>NS</td>
</tr>
<tr>
<td>*FHx of IHD (N, %)</td>
<td>7 (43%)</td>
<td>57 (40.7%)</td>
<td>NS</td>
<td>48 (69.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking (N, %)</td>
<td>7 (43%)</td>
<td>47 (33.8%)</td>
<td>NS</td>
<td>40 (58%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*FHx of IHD = Family history of ischemic heart disease

Study overview of Immuno-inflammatory response

Patients with CAE (16 patients, mean age 64.9 ± 7.3 years, 6 female) were compared with the matched controls (140 patients, mean age 58.6 ± 4.1 years, 40 female). Systemic levels of INF-γ, TNF-α, IL-1β, and IL-8 were significantly higher in CAE group compared to control and CAD groups using Kruskal-Wallis H, while the levels of IL-2 and IL-4 were lower (P<0.001 for both) compared to the same groups. No significant differences were found in...
the systemic levels of cytokines IL-10, IL-12P “subunits IL-12 and IL-23”, and IL-13 between all groups (Table 11 and Figure 8).

Table 11: Immuno-inflammatory response and related cytokines levels in the CAE group vs control group and CAD patients. Data is presented as median (interquartile range) and p-values is derived from Kruskal–Wallis H-tests, using Dunn’s test with Bonferroni correction for post-hoc tests.

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Controls (n = 140)</th>
<th>CAE Patients (n = 16)</th>
<th>p-Value CAE vs. Control</th>
<th>CAD (n = 69)</th>
<th>p-Value CAD vs. CAE</th>
<th>p-Value Kruskal-Wallis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I-Equal Levels (median and interquartile)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg./mL)</td>
<td>0.25 (0.15)</td>
<td>0.26 (0.11)</td>
<td>-</td>
<td>0.29 (0.21)</td>
<td>-</td>
<td>0.07</td>
</tr>
<tr>
<td>IL-12p (sub-unit)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-12 and IL-23</td>
<td>0.09 (0.11)</td>
<td>0.08 (0.08)</td>
<td>-</td>
<td>0.09 (0.09)</td>
<td>-</td>
<td>0.41</td>
</tr>
<tr>
<td>(pg./mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-13 (pg./mL)</td>
<td>0.26 (0.60)</td>
<td>0.14 (0.44)</td>
<td>-</td>
<td>0.28 (0.66)</td>
<td>-</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>II-Reduced Levels (median and interquartile)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2 (pg./mL)</td>
<td>0.25 (0.08)</td>
<td>0.12 (0.05)</td>
<td>&lt;0.001</td>
<td>0.26 (0.11)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-4 (pg./mL)</td>
<td>0.04 (0.03)</td>
<td>0.004 (0.006)</td>
<td>&lt;0.001</td>
<td>0.04 (0.03)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>III-Increased Levels (median and interquartile)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg./mL)</td>
<td>0.64 (0.44)</td>
<td>0.98 (0.60)</td>
<td>0.049</td>
<td>0.73 (0.48)</td>
<td>0.25</td>
<td>0.046</td>
</tr>
<tr>
<td>IL-8 (pg./mL)</td>
<td>3.62 (2.18)</td>
<td>5.59 (3.65)</td>
<td>0.007</td>
<td>3.82 (2.30)</td>
<td>0.023</td>
<td>0.009</td>
</tr>
<tr>
<td>IFN-γ (pg./mL)</td>
<td>3.88 (2.55)</td>
<td>5.45 (3.33)</td>
<td>0.032</td>
<td>4.72 (4.58)</td>
<td>0.74</td>
<td>0.006</td>
</tr>
<tr>
<td>IL-1β (pg./mL)</td>
<td>0.001 (0.01)</td>
<td>0.17 (0.24)</td>
<td>&lt;0.001</td>
<td>0.00 (0.05)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>TNF-α (pg./mL)</td>
<td>1.82 (0.71)</td>
<td>2.37 (0.74)</td>
<td>0.002</td>
<td>1.96 (0.66)</td>
<td>&lt;0.12</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 8. Cytokines with significantly higher levels in CAE patients compared to controls. P-values are derived from Kruskal–Wallis test of all three groups (see Table 2). CAE patients also presented with significantly higher levels IL-1β and IL-8 than CAD patients. Boxes show median and interquartile. Dashed lines indicate the threshold for defining extreme values, which are shown in a compressed region between the solid lines.

**Pure ectasia vs. mixed ectasia**

Cytokine levels were comparable between patients with pure (n=6) and mixed (n=10) CAE. Although, IL-4 showed lower trends in the mixed CAE group (0.004 vs 0.007 pg./L), yet this was not significant (p=0.06) (Table 12)
Table 12. Immuno-inflammatory response in CAE sub-groups of pure and mixed. Data are presented as median (interquartile range; IQR) and p-values derived from Mann Whitney U-tests.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mixed Ectasia (n= 10)</th>
<th>Pure Ectasia (n=6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 (pg./ml)</td>
<td>0.27 (0.12)</td>
<td>0.25 (0.19)</td>
<td>0.79</td>
</tr>
<tr>
<td>IL-12p (sub-unit IL-12 and IL-23 (pg./ml))</td>
<td>0.07 (0.07)</td>
<td>0.11 (0.12)</td>
<td>0.64</td>
</tr>
<tr>
<td>IL-13 (pg./ml)</td>
<td>0.18 (0.57)</td>
<td>0.14 (0.37)</td>
<td>0.71</td>
</tr>
<tr>
<td>IL-2 (pg./ml)</td>
<td>0.15 (0.07)</td>
<td>0.12 (0.03)</td>
<td>0.15</td>
</tr>
<tr>
<td>IL-4 (pg./ml)</td>
<td>0.004 (0.004)</td>
<td>0.007 (0.012)</td>
<td>0.06</td>
</tr>
<tr>
<td>IL-6 (pg./ml)</td>
<td>0.99 (1.06)</td>
<td>0.94 (0.51)</td>
<td>0.49</td>
</tr>
<tr>
<td>IL-8 (pg./ml)</td>
<td>5.67 (3.49)</td>
<td>4.79 (3.67)</td>
<td>0.37</td>
</tr>
<tr>
<td>IFN-γ (pg./ml)</td>
<td>5.39 (3.10)</td>
<td>5.96 (6.25)</td>
<td>0.26</td>
</tr>
<tr>
<td>IL-1β (pg./ml)</td>
<td>0.22 (0.37)</td>
<td>0.13 (0.17)</td>
<td>0.49</td>
</tr>
<tr>
<td>TNF-α (pg./ml)</td>
<td>2.37 (0.73)</td>
<td>2.22 (0.97)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Qualitative assessment and assessment of accuracy and reproducibility

The methodological strengths of the results were owed to many factors: First, plasma samples of both groups were run in duplicate. All the measured quantities were compared between both runs and no significant differences between both sets of results was noted. This confirmed the accuracy of the quantitative results. Second, for biochemical analysis, the advantage of using highly sensitive multiplex multiphoton ELISA test has facilitated uniform quantification of all markers at the same time. Unlike former methods that might require many samples with different sample preparations - and hence multiple apparatus calibrations - that would play a potential role for incidences of minor quantitative errors. Finally, the chosen method was already proven to be with high sensitivity to detect lower concentrations, however, the lowest detection limit (LLOD) was calculated according to the manufacturers’
protocol and the mean value for the three plates was used for further calculation of the sample concentrations. Any value below the lowest limit of detection for the cytokine assay was replaced with zero in the statistical calculations.

**Paper Four:**

**Demographics and CV risk factors**

Demographics baseline, CV risk factors, MACE and cardiovascular mortality over the follow up period were examined in the CAE and control groups and compared in (Table 13). Controls were matched with CAE patients in gender, hypertension, hypercholesterolemia and diabetes mellitus; however, controls were slightly younger and had shorter follow up. The prevalence of family history of CAD and smoking were significantly higher in CAE (p=0.02 and p=0.001, respectively). On the other hand, CAE patients had higher CV mortality (p=0.03) but the same readmission rate with MACE (p=0.26) (Table 13).
Table 13: Demographic and cardiovascular comparison between patients and controls.

CAE, coronary artery ectasia   CAD, coronary artery disease

<table>
<thead>
<tr>
<th></th>
<th>Controls (n 41)</th>
<th>CAE (n 41)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, median IQR)</td>
<td>61 (56-68)</td>
<td>66 (60-74)</td>
<td>0.003</td>
</tr>
<tr>
<td>Gender (F, %)</td>
<td>12 (29.3 %)</td>
<td>11 (26.8%)</td>
<td>0.81</td>
</tr>
<tr>
<td>Risk Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>24 (58.5 %)</td>
<td>22 (53.7 %)</td>
<td>0.82</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>8 (19.5 %)</td>
<td>7 (17.1 %)</td>
<td>0.78</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>15 (36.6 %)</td>
<td>30 (73.2 %)</td>
<td>0.001</td>
</tr>
<tr>
<td>Dyslipidemia (n, %)</td>
<td>22 (53.7 %)</td>
<td>27 (65.6 %)</td>
<td>0.26</td>
</tr>
<tr>
<td>Family History for CAD (n, %)</td>
<td>12 (29.3 %)</td>
<td>22 (53.7 %)</td>
<td>0.02</td>
</tr>
<tr>
<td>Overall Mortality (n, %)</td>
<td>0</td>
<td>9 (22 %)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cardiovascular Mortality (n, %)</td>
<td>0</td>
<td>5 (12.2 %)</td>
<td>0.03</td>
</tr>
<tr>
<td>* MACE (n, %)</td>
<td>13 (31.7 %)</td>
<td>18 (43.9 %)</td>
<td>0.26</td>
</tr>
<tr>
<td>Follow-up period (year, median IQR)</td>
<td>10.0 (9.7 – 10.3)</td>
<td>11.4 (10.1 – 12.2)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*MACE: major acute cardiac events

CV profile of CAE mortality

CV mortality was documented in 5/41 patients (Figure 9 A), representing 12% of the CAE cohort. There was no significant difference in overall survival in relation to demographics or CV risk factors (p>0.05) (Table 14). CAE mortality was related to ventricular arrhythmia in 2 patients. Atrial fibrillation complicated by stroke is found in one patient. Two patients with history of excess Alcohol developed dilated cardiomyopathy and heart failure (Figure 9 B). Apparently, all CAE non-survivors smoked and had dyslipidemia. The trend of differences between these subgroups was noticeable, but not significant.
Table 14: Cardiovascular risk factors comparison between CAE survivors versus cardiovascular mortality

<table>
<thead>
<tr>
<th></th>
<th>CV Mortality (n=5, 12.2%)</th>
<th>Survival (n=36, 87.8%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, median (IQR))</td>
<td>68 (59-73)</td>
<td>72 (68-75)</td>
<td>0.16</td>
</tr>
<tr>
<td>Gender (female, %)</td>
<td>1 (20%)</td>
<td>10 (27.8 %)</td>
<td>0.59</td>
</tr>
<tr>
<td>Risk Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>3 (60.0%)</td>
<td>19 (52.8%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>1 (20.0%)</td>
<td>6 (15.4%)</td>
<td>0.63</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>5 (100%)</td>
<td>25 (69.4%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Dyslipidemia (n, %)</td>
<td>5 (100%)</td>
<td>22 (61.1%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Family history for CAD (n, %)</td>
<td>2 (40.0%)</td>
<td>20 (55.6%)</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Figure 9- A Overall cardiovascular mortality (8A) and MACE according to presentations in CAE patients (9- B). AF = atrial fibrillation, CVA= cerebrovascular events, DCM= dilated cardiomyopathy, ACS= acute coronary syndrome, CABG= Coronary artery bypass graft and MACE= Major acute cardiac events
CV profile of patients with MACE

MACE and CV mortality were documented in 18 CAE patients (44 %) (Figure 8B) during the follow up period, with 34% (14 out of 18) survival. Most events were related to the development of atrial fibrillation (4 patients, additional patient with cerebrovascular event with no clear documentation of AF at presentation and hence not categorized), ACS or acute MI (3 patients), urgent coronary artery bypass surgery (2 patients), DCM (2 patients) with heart failure and cardiac arrest in (2 patients) who presented primarily with ventricular arrhythmias (Figure 8B). Patients who developed MACE were relatively older (p=0.09), mostly females (8 patients, p=0.03) and have no family history for CAD (6 vs. 16, p=0.03). The other CV risk factors were not different between groups (Table 15).

Table 15: Comparison between CAE patients with and without MACE.
MACE= major acute cardiac events.

<table>
<thead>
<tr>
<th></th>
<th>MACE* (n 18, 43.9%)</th>
<th>No MACE (n=23, 56.1%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, median (IQR))</td>
<td>70 (64-75)</td>
<td>65 (58-72)</td>
<td>0.09</td>
</tr>
<tr>
<td>Gender (female, %)</td>
<td>8 (44.4%)</td>
<td>3 (13.0 %)</td>
<td>0.03</td>
</tr>
<tr>
<td>Risk Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>12 (66.7%)</td>
<td>10 (45.5%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>4 (22.2%)</td>
<td>3 (13.0 %)</td>
<td>0.36</td>
</tr>
<tr>
<td>Smoking (n, %)</td>
<td>15 (83.3%)</td>
<td>15 (65.2%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Dyslipidemia (n, %)</td>
<td>11 (61.1 %)</td>
<td>16 (69.6%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Family history for CAD (n, %)</td>
<td>6 (33.3 %)</td>
<td>16 (69.6%)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*MACE: Major acute cardiac events
Discussion

This thesis has focused on CAE pathophysiology emphasising on its clinical presentations and prognosis. Rather than historical data, we presented the true prevalence of CAE in patients with acute coronary syndrome - requiring urgent coronary revascularization “i.e. PPCI presentations” - and its inflammatory response with related short-term prognosis. Furthermore, we used a novel method of identifying personalized lipid metabolic profile and metabolic pathways in CAE in an attempt to separate it from commonly liked atherosclerotic CAD. However, believing in the chronic inflammatory response of CAE, we have examined an extended panel of cytokines systemic levels to investigate the immune inflammatory response of the disease. This study enhances the previous studies in showing a relatively different pathogenesis from atherosclerosis. Finally, we concluded by investigations of long-term outcome of CAE including MACE and Cardiovascular mortality rates.

1) Coronary ectasia in acute myocardial infarction

In this study, we found that the incidence of CAE, among a cohort of patients presenting with acute MI who underwent a PPCI procedure, was 2.7%. Conventional CV risk factors, except DM, did not differentiate between patients with and without CAE. Patients with CAE had less number of identified culprit lesions and the RCA and LCX, as previously shown, were predominantly involved compared to patients without CAE. Inflammatory markers concentrations were significantly different between groups, with higher levels of CRP and lower WCC, neutrophil level and N/L ratio in patients with CAE, particularly with diffuse disease, when compared to patients without CAE and those who had complete arterial occlusions. Nevertheless, the inflammatory markers levels did not add any prognostic value
between CAE and non-CAE patients, and did not influence the management options (medical treatment vs intervention).

**Characteristics of PPCI presentation in coronary ectasia**

The study investigated patients with ACS required PPCI and compared those with and without CAE. Anatomical findings revealed that patients with CAE had more frequent involvement of the RCA and LCX. Moreover, CAE had diffuse involvement of the coronary arteries (i.e. more than a single artery involved). Ectatic arteries with MI had evidence of more exaggerated inflammation with higher inflammatory markers levels. However, risk factor profiles were similar in both groups, except DM that was independently and inversely associated with CAE [63, 109], in contrast to atherosclerotic CAD [122]. Our observation is similar to those studies, that showed DM prevalence was lower in CAE. Therefore, we hypothesize that the pathophysiology of CAE is likely to be different, even if partially, from that of atherosclerosis.

Moreover, the 2-year clinical follow-up data did not show any difference in MACE rates between CAE and atherosclerosis patients. Coronary ectasia did not advocate any worse outcomes to the acute MI in this small cohort in agreement with what was previously found [7]. The similar clinical outcome suggests that acute MI presentations were not influenced by the presence of additional CAE. Furthermore, standard inflammation accompanying the two groups, although different, failed to predict the clinical outcome in either group. Our findings suggest that acute MI presentation entailed the standard treatment options, thus resulting in similar short-term clinical outcomes in both groups. Finally, the current results cannot explain whether the exaggerated inflammatory response at presentation is a potential cause for ACS or just purely an association with CAE.
The findings of this paper have clinical implications having showed that acute MI patients with CAE have an increased inflammatory response. A similar finding was observed in acute thrombotic lesions in acute MI with a sharp rise (within a few hours) in the WCC and hence the N/L ratio (as lymphocytes are not affected by acute lesions), when compared to stenotic, non-obstructed coronary lesions. The increased inflammatory response of the N/L and neutrophils was associated with localized CAE (i.e. including one segment in one coronary artery), but not with diffuse CAE. Consequently, a different pathophysiology of the two conditions can be suggested as has previously been proposed; with localized CAE representing heavy inflammation secondary to aggressive atherosclerosis, plaque rupture and thrombus formation; however, CAE may have primarily induced recurrent ischemia by CSF and minor ischemic culprits rather than the total artery thrombosis [3, 7]. Finally, the presence of CAE in acute MI presentation does not seem to have a direct effect on the clinical outcome and choice for interventional or medical revascularization.

2) Personalized lipids profiling in coronary ectasia

The exact pathophysiology of CAE has not been well elucidated. The disease is either well correlated with a heavy CAD atherosclerotic burden in the West or is related to the inflammation of the coronary tree in the East (e.g. Kawasaki diseases, autoimmune arteritis…etc.) [123]. Despite this common associations, histopathological evaluation suggests a different pathological process including fatty degeneration and hyalinization of the arterial wall, hence aneurysmal formation [58]. This study presents a comprehensive analysis of lipid metabolites and its dysregulation in patients with CAE. This was performed by employing a new method of analysis of the metabolic profile and untargeted lipidomic analysis “Metabolomics/ Lipidomics analysis”. We extracted the identifiable metabolites in significant concentrations in CAE; this in turn reflects the metabolic process and explains the
possible dysregulated pathways. Our results demonstrate the potential ability of metabolic profiling in providing markers that could feature coronary ectasia. To our knowledge, this is the first extended lipid profiling application in CAE disease.

➢ Metamorphoses in coronary ectasia Lipids profile

The main finding of this study is the dysregulation of sphingomyeline (SM) and phosphatidylcholine (PC) in CAE, representing a potentially different metabolic process compared to controls. SMs and PCs were significantly different between the two groups; Twenty-seven metabolites were identified, of which, sixteen metabolites were recognized as PC species and another eleven as SM species. All quantitative assessments and revalidation tests confirmed the significance of the lower concentrations of these species in the CAE group. However, the limited results of three metabolites that differed between the pure CAE and mixed CAE were two SM metabolites and one PC. Of note, the value of signalling lipids in phosphoinositides, sphyngolipids, and fatty acids in controlling important cellular processes, of atherosclerosis, such as cell proliferation, apoptosis, metabolism and migration have been established [124].

A) Phosphatidylcholine metabolites in coronary artery ectasia

In general, PC metabolites play an important role in the atherosclerosis process where they carry fatty acids and promote lipid deposition in the arteries. This behaviour has previously been confirmed in CAD, when PC levels were consistently elevated, as well as in acute coronary syndrome [124]. The down regulation and lower levels of PC species in patients with CAE may suggest a different metabolic process, thus indicating a different metabolic pathophysiology to that of atherosclerosis.
B) Sphingomyelin metabolites in coronary artery ectasia

SM species are carried into the arterial wall on atherogenic lipoproteins, where they get engulfed by macrophages and monocytes in foam cell formation. This represents an early spark of atherosclerosis and plaque formation. They are also separated from atherosclerotic plaques in addition to the polyunsaturated cholesteryl esters with long-chain fatty acids [24]. Human plasma SM levels are positively correlated with CAD development; their levels could be a marker for atherogenic lipoprotein accumulation and hence a heavy atherosclerotic burden in the coronaries [25]. The potential role of SM species in the atherosclerotic process and plaque formation would suggest that the lower concentrations of SMs in CAE may be attributed to a different non-atherogenic pathway of lipid metabolism. It can also be postulated that the sphingomyelinase enzymes are triggered in CAE, which activates apoptotic processing of the arterial wall. This eventually leads to arterial wall weakening, dilatation and then become ectatic [124, 125].

➢ Dysregulated lipid profile with minimal atherosclerosis

In addition to the 27 metabolites dysregulated in the CAE group, we also separated another three metabolites between patients with pure CAE and mixed CAE with very minimal evidence of atherosclerosis. These species are SM (d18: 1/16:0), phosphocoline (d18: 2/17:0), and SM (d18: 1/24:2), which had significantly lower levels in patients with mixed CAE than in those with pure CAE and controls (Figure 3). However, we noticed another 13 metabolites (10 SM and 3 PC) which tended to be higher in pure than in mixed ectasia, but these differences did not reach statistical significance, perhaps because of the small studied number. The conceivable description of the lower intensities of PCs in the mixed CAE group is due to its transformation into arachidonic acid as donors of fatty acyl chains (FACs). This
may contribute to the early atherosclerotic process in mixed CAE and hence the PCs species are in lower quantities [126].

Lower SM intensities in patients with mixed CAE is rather complex. The Ceramid pathway activation for apoptotic processes may infrequently be resisted by phosphorylation by Ceramid kinase enzyme. This process promotes an active inflammatory response predisposing to atherosclerosis and may partially explain the subgroup of patients with mixed CAE [124]. However, this interpretation should be taken cautiously because of the generally similar nature of pure and mixed CAE disease and also the small number of patients with pure CAE we studied.

3) **Immuno-inflammatory response in coronary artery ectasia**

Systemic immune-inflammatory status was investigated in patients with CAE. The systemic levels of INF-γ, TNF-α, IL-1β, IL-6, and IL-8 cytokines were higher in the CAE patients; conversely, the levels of IL-2 and IL-4 were lower than CAD and controls. The cytokine panel triggered here may suggest a state of increased macrophage activation. On the other hand, cytokines levels were comparable between CAE sub-groups of patients, with pure and mixed CAE. The latter would be expected, as the levels of atherosclerosis were minimal and CAE is the main insult in those coronaries not atherosclerosis.

![Pro-inflammatory response of coronary artery ectasia](image)

Atherosclerotic obstructive CAD and CAE have been found to have predominantly higher systemic levels of IL-6 when compared to normal variants as reported by Triantafillou *et al.* They also reported higher levels of IL-4, and lower levels of IL-2 in patients with CAE compared with those of obstructive CAD. This pro-inflammatory response could suggest the involvement of TH2 immune pathway activation in CAE [67], as similarly been proposed by...
Adioglu et al. [68]. It is well established that TNF-α plays a key role in the process of atherosclerosis through stimulation of the TH1 pathway, thus leading to macrophage activation [127]. However, previously reported lower TNF-α levels in patients with CAE [68, 128] may argue also against TH1 pathway activation in CAE, thus representing another difference from the atherosclerotic inflammation process.

❖ Cytokines profile in coronary artery ectasia

The systemic immune-inflammatory status in CAE patients show a different cytokine milieu compared to the CAD and controls. The levels of cytokines in CAE (IL-6, IL-8, IFN-γ, IL-1β, and TNF-α), were higher when compared to normal and CAD. In atherosclerosis CAD, TH1 enhances macrophage activation through IFN-γ and IL-2. The pro-inflammatory response in CAE is exaggerated when the same biomarkers are compared with atherosclerosis (higher cytokines systemic levels). Nevertheless, this study indicated lower levels of IL-2 in patients with CAE, which may suggest attenuated positive feedback of macrophage activation unlike atherosclerosis [127]. This correspondingly may reinforce the notion of a non-atherosclerotic process in CAE. Such finding also establishes an interesting link with our previously published lipidomic profiling data, which showed lower SM levels in patients with CAE than in controls. The low SM levels reduce lipoprotein aggregation - engulfed by activated macrophages- and hence less macrophage foam cells, the main component in atheroma. The result is less atheroma formation and burden [80, 127].

❖ IL-6 role in coronary artery ectasia

The role of elevated IL-6 level in atherosclerosis process is debateable [129, 130]. While IL-6 can be regarded as a pro-inflammatory cytokine, it may also reduce the activity of pro-inflammatory cytokines by inhibiting macrophage scavenger receptor-A, thus reducing
macrophage activation [131]. Similarly, another study showed that IL-6 levels did not correlate with an increased risk of acute ischemic heart events [132]. These findings suggest that a lower pro-inflammatory response and reduced macrophage activation is enough to dispute the atherosclerotic nature in CAE group.

- **IL-4/TH2 role in coronary artery ectasia**

TH2 cells secrete IL-4, helps antibody production by B cells and wound healing macrophage activation (M2) [133]. The TH2 lineage is not recognized within atherosclerotic lesions and hence the IL-4, its prototype-related cytokine, does not significantly influence the development of atherosclerotic lesions and lower IL-4 levels were associated with atherosclerosis regression [134]. IL-4 levels were low in CAE patients and these findings also can partially dispute against an atherosclerotic process in CAE.

- **Pro-inflammatory pathophysiology and macrophage heterogeneity in coronary ectasia**

The heterogeneous macrophage activation results in the production of various cytokines, which have complex interactions [135, 136]. The main three populations of activated macrophages (M1- M3) are known as classical macrophages, wound-healing macrophages, and regulatory macrophages [133]. Conventionally, cell-mediated immune response activates macrophages leading to TH1 cell implication. This response is recognized in atherosclerosis process. Moreover, TNF-α and IFN-γ enhance macrophage activation, leading to higher levels of pro-inflammatory cytokines and mediators. This can cause extensive damage to the host (i.e. arterial wall intima) and lead to atherosclerosis and hence thrombosis [67, 137]. The immune inflammatory response in CAE yields increased IL-1β, IL-6, TNF-α, and IFN-γ. Classic “tissue” macrophage activation in response to innate stimuli might occur in response
to a trigger of stress or viral infections in patients with CAE, in addition to IFN-γ. This trigger may play a role in compensating for the attenuated IL-2 levels secreted by TH1. However, the main trigger for the pro-inflammatory status remains unidentified.

❖ Cytokines role in the pathophysiology of coronary artery remodelling

The elevated cytokines levels in CAE may reflect an exaggerated response to enzymatic degradation of the extracellular matrix of the media producing extensive vascular remodelling [36, 138]. Histological studies have confirmed this nature of the extensive destruction of the musculo-elastic element, with marked degradation of the medial collagen and elastin fibres, and disruption of the internal and external elastic lamina [74, 139]. In addition, the elevated immune inflammatory markers, TNF-α and IFN-γ in our patients with Coronary ectasia are known to have a broad range of activated MMPs that inhibit collagen synthesis. These effects are mediated by leukocyte transmigration and local cytokine activation of macrophages. Cell-surface adhesion molecules have been found to be elevated in CAE populations with no atherosclerosis [133, 140]. Combining this with reduced IL-4 (i.e., reduced adaptive immune response by reducing wound healing macrophages, hence promoting unopposed tissue damage) [141] might suggest the heightened destructive vascular remodelling with CAE.

❖ Pure vs mixed coronary ectasia cytokines response

Our results showed no difference in the pro-inflammatory systemic response between pure and mixed ectasia. Despite of a slightly higher trend of increased IL-4 systemic levels in pure CAE was not statistically significant (p = 0.06), no other observations of significance
between both groups. The study cohort remains relatively small but comparable levels support the theory.

4) Long term outcome of coronary artery ectasia

A relatively long-term follow up for more than 10 years on patients with CAE showed CV related mortalities were significantly higher in CAE patients when compared to controls.

a) Cardiovascular related Morbidity and mortality in coronary artery ectasia

Overall CV-related mortality rates were significantly higher in CAE patients compared to controls. Despite the fact that CAE patients were slightly older, they smoked and had stronger family history for CAD than controls. Apart from smoking in the CAE group, the rest of the conventional CV risk factors were not different from controls. Overall, CV risk profile failed to predict MACE or mortality among CAE patients in a sub analysis, probably because of the small study cohort.

b) Coronary artery ectasia prognosis

Adverse cardiac events are well established consequence of CAE despite the benign nature of pure CAE without atherosclerosis [142]. However, the literatures about CAE follow up revealed different outcomes with variable study configurations (e.g. shorter periods of follow up or investigating a mixed atherosclerosis with CAE). A three-years follow up of CAE carried similar clinical outcome even when CAE was associated with heavy atherosclerosis burden [110]. Likewise, other studies reported non-benign course in CAE as a result of dilated lumen with disrupted flow, a known substrate for potential thrombus formation [48, 112, 143]. In our study, we have investigated the non-atherosclerotic forms of CAE clinical course for a longer follow up period. The results showed patients with CAE who had no
significant atherosclerotic coronary stenosis having higher mortality, CV mortality and trends towards higher prevalence of hospital re-admissions with chest pain, acute coronary syndrome and arrhythmia. The previously suggested slow coronary flow phenomena may explain the inferior clinical outcome.

We reported in our patients the development of dilated cardiomyopathy with heart failure in two patients, as previously documented [144]. Perhaps the long-term outcome in our study provides a stronger evidence for worse clinical outcome in CAE compared to controls.

c) **Coronary artery ectasia cardiovascular risk profile and MACE**

Conventional CV risk factors showed by Ipek G et al that smoking was independently associated with CAE-related MACE, particularly ACS and MI [83]. Our study may support this finding, having shown that smoking and dyslipidaemia carried higher trends toward higher mortality in CAE. Most of the rest of CV risk factors (except family history for IHD and female gender) were similar between CAE patients with and without MACE, thus refuting the potential impact of risk factors on MACE in such patients. Similarly, the remainders of CV risk factors did not seem to play any role in CV mortality in the same group of patients. This may support our previous suggestion that CAE (particularly non-atherosclerotic) is quite different from conventional atherosclerosis [80, 145]. Similarly, the prognosis of CAE is different with worse long-term outcome [83, 144].
Limitations


This is a retrospective case-control study and so is subject to the known limitations and potential bias. Data were collected from hospital clinical records, which are believed to be accurate. Therefore, we examined the conventional inflammatory markers only as blood samples were out-dated for further analysis of potentially specific markers. However, CRP is a reliable marker and has been shown previously to correlate with IL-6 in isolated patients with CAE (14). The definition of CAE in this study was based on criteria of an ectatic part length is more than 20 mm in addition to the same other criteria (i.e. to avoid recruiting focal aneurysmal dilatation that is a variant of heavy atherosclerosis). We did not use one criterion of lesions more than 1/3 of the artery length to increase the sensitivity of our criteria to recruit more patients and apparently, the longer segment (i.e. more than 20 mm) was adequate to indicate true CAE.

This paper results failed to answer the clinical question, “is CAE a part of the atherosclerotic process or an independent pathology?” since we did not have any previous coronary angiograms to diagnose CAE before acute presentations but the dissimilar inflammatory response may partially indicate the differences. The small number of study cohort is another limitation to this relatively rare condition, however, we enrolled a double number of age/sex-matched controls to empower the statistical analysis and results.
Paper 2: Dysregulated fatty acid metabolism in coronary ectasia: An extended lipidomic analysis

The goal of this study was to establish a preliminary pattern of metabolic disturbances that characterise CAE. Our results are interpreted in the context of the available clinical and biochemical data with no significant restrictions on diet for patients or controls. Although the risk factor profile did not differ between CAE patients and controls, little is known about the exact duration that these risk factors exist for. In addition, the intake of statins “which is commonly administered’ may have influenced the metabolic profile. Finally, the small studied number, in particular those with pure CAE, limits the generalisation of our findings but this study remains the first to examine, in depth, the lipid metabolism in CAE patients.

Paper 3: Cytokine disturbances in coronary artery ectasia do not support atherosclerosis pathogenesis.

Due to a relatively rare prevalence of pure CAE the sample size was relatively small. However, we have randomized this against larger control cohort to add power to statistical outcome and results. The difficult geographical distribution of the Swedish cohort stood partially against obtaining blood samples from all of the 49 original identified patients with CAE. Despite of patients were recruited from two different countries; Sweden and Ireland; however, this cohort is relatively homogeneous representing white Caucasian and northern European patients predominantly.

Study 4: Coronary artery ectasia carries worse prognosis: a long-term follow-up study

Despite the long term follow up of our study patients using strict criteria for, the already rare, CAE diagnosis, the cohort was rather small, especially those with complete follow up data. The latter may limit the relevance of statistical findings. The control group seems relatively
younger, but this is indebted to specific coronary anatomy criteria of having only minimal
disease and minimal atherosclerosis burden. Despite controls were relatively younger, yet
they were followed up for a long period of more than 10 years to assure the relevance of the
results. Some differences may exist between patients and controls in terms of individual
habits and life style that may lead to a limited impact.
Conclusion

**Paper 1**

In acute MI, patients with CAE commonly have CAE changes in the RCA and LCx, and higher inflammatory markers compared to those without CAE. However, these differences did not have any short-term prognostic impact to suggest altered management.

**Paper 2**

This lipid profiling study demonstrated dysregulation and reduced levels of SM, which suggests significant premature apoptosis in CAE and hence arterial wall dilatation. Additionally, the lower PCs concentrations in CAE would suggest perturbations in fatty acid elongation/desaturation; this may postulate the non-atherogenic nature of CAE.

**Paper 3**

The enhanced systemic pro-inflammatory response in patients with CAE highlights the study findings. The profile of the CAE immuno-inflammatory response might suggest macrophages activation consequential to a possible viral or stress trigger. The CAE cytokine milieu differs from that of atherosclerosis indicating a different pathophysiology despite the common chronic inflammatory process. Further studies are warranted to validate these findings.

**Paper 4:**

Coronary artery ectasia prognosis is serious and has worse prognosis with higher cardiovascular mortality and trends towards increased MACE readmissions than others with matched minimal CAD. Among CAE patients, older females had higher mortality. Smoking and dyslipidaemia seem to have important prognostic role.
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