Tampereen yliopisto

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A 50–YEAR AUTOPSY BASED STUDY APPROACH WITH CORONARY MEASUREMENTS ON EPIDEMIOLOGY, PATHOLOGY, RISK FACTORS AND GENETICS OF SUDDEN CARDIAC DEATH

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Syyskuu 2020

TIIVISTELMÄ

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Oikeuslääketieteen tutkimusryhmä

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Tässä työssä kokosimme Helsingin ja Tampereen yliopistojen oikeuslääketieteen laitoksilla 60-luvun lopulta asti kerätyt sepelvaltimotaudin ja sydänäkkikuoleman tutkimiseen tarkoitetut ruumiinavausaineistot yhdeksi kohortiksi. Aineisto koostuu kaikkiaan viitenä eri vuosikymmenenä kerätystä aineistosta, jossa on yhteensä 2715 erillistä ruumiinavaustapausta.

Kaikki kohorttissa olevat tapaukset ovat sairaalan ulkopuolella tapahtuneita äkillisiä kuolemantapauksia, joille on suoritettu täysi oikeuslääketieteellinen ruumiinavaus ja näihin liittyvät normaalit toksikologiset ja mikroskooppiset analyysit. Lisäksi aineistoon on kaikissa tapauksissa kerätty sepelvaltimoiden ja muiden keskeisten valtimoiden tautimuutosten morfologiset mittaukset mukaan lukien ahtauman määritys sekä plakkien tyypitys ja pinta-alat. Osassa avaussarjoissa on mukana myös geneettiset tutkimukset, riskitekijöiden kartoitus läheisten haastattelulla sekä erilaiset mikrobiologiset ja muut näytteet.

Vaikka sepelvaltimotautikuolemien määrä on viimeisen 40 vuoden aikana vähentynyt merkittävästi länsimaissa, ovat sydän- ja verisuonitaudit maailmanlaajuisesti edelleen merkittävin kuolinsyy ja niiden määrä kehittyvissä maissa on nousussa. Vuonna 2015 niihin kuoli arviolta 17,7 miljoona ihmistä, mikä vastaa 31% kaikista kuolemista. Myöskään nuorten kohdalla sepelvaltimotautikuolleisuudessa ei ole ollut yhtä merkittävää laskua kuin vanhemmissa ikäluokissa. Muutokset ravinnossa, tupakoinnin väheneminen ja muutokset hoidossa mm. ACE-estäjien, ATR2-salpaajien ja ohitusleikkauksien muodossa, ovat muuttaneet sepelvaltimotaudin kliinistä kuvaa. Tällä hetkellä äkillisestä sairaalan ulkopuolella tapahtuvasta kuolemasta onkin tullut sepelvaltimotautipotilaan yleisin kuolemantapa.

Äkkikuolemien pohjalta tehdyt ruumiinavaukset mahdollistavat laajojen, hyvin koko väestöä vastaavien aineistojen, keräämisen sepelvaltimotautikuolemaan liittyen. Lisäksi suomalaisen lainsäädännön takia oikeuslääketieteellisten ruumiinavausten määrä on korkea ja kohortissa olevat tapaukset kattavat n. 20% kaikista niiden keräys aikana tapahtuneista kuolemista. Kerätty aineisto tarjoaakin ainutlaatuisen mahdollisuuden sepelvaltimotaudin epidemiologian ja patofysiologian tutkimiseen.

Avainsanat: sepelvaltimotauti, sydänäkkikuolema, sydäninfarkti

Table of contents

Why was the cohort set up? 4
Who is in the cohort? 4
What has been measured? 7
What has it found? Key findings and publications 11
What are the main strengths and weaknesses? 13
Can I get hold of the data? Where can I find out more? 13
Acknowledgements 14
References 14

Why was the cohort set up?

Although coronary heart disease (CHD) mortality rates have fallen dramatically over the past 40 years in the Western world, cardiovascular diseases (CVD) are still the most common cause of death globally, showing an increase in developing countries (1). Approximately 17.7 million people died from CVDs in 2015, representing 31% of all global deaths (1). Of these deaths, an estimated 7.4 million were caused by CHD and 6.7 million due to myocardial infarction (MI) (1). While CHD death rates in older populations have been falling steeply e.g. in the USA, there has been a smaller decrease among young adults since 1990 (2). Moreover, due to the better medical care of in-hospital MI patients, unpredictable sudden out-of-hospital death has become the most frequent way of death of a CHD patient (3). All these figures suggest that the burden of CVD and especially CHD continues to be in the hotspot of health care and that there is a need to find new and better prevention and treatment strategies.

Due to emerging data on significant differences in the mortality from coronary heart disease (CHD) between populations and countries in the 1950s, research interest rose to study possible population-specific differences in the severity of atherosclerosis. The most comprehensive effort culminated in International Atherosclerosis Project in 1960-1965 in which autopsy coronary samples from over 20 000 individuals in 19 different countries were studied using a visual scoring method. Finland was not among the countries participating in the study. During the 1960s, Finland was one of the leading countries in CHD mortality and Finnish males aged under 65 had the highest CHD mortality rate in the World (4). This knowledge was the main background for starting to collect the first prospective autopsy series in Finland in 1968 (5).

During 50 years four independent autopsy studies were collected in five different decades from the late 1960s to 2010s covering altogether 2715 autopsy cases (Table 1). This study project has up to now yielded 17 medical theses and more than 200 articles in referenced journals. As the most important feature, all these autopsy series are based on coronary measurements at the vessel wall level. This unique approach enables researchers to have a comprehensive view on the epidemiology, risk factors, pathology and genetics of CHD and its most feared consequence out-of-hospital sudden cardiac death (SCD). During this time span, the cardiovascular disease management has improved dramatically by the development of effective medical therapies such as statins, ACE-inhibitors and ATR2 blockers as well as interventions such as coronary artery bypass grafting as well as percutaneous coronary intervention. The focus for collecting the series has shifted from epidemiology and risk factors of coronary atherosclerosis to the genetics of SCD and inflammation of the coronary plaque.

Who is in the cohort?

In Finland, one of the advantages was that Finnish legislation enabled the collection of prospective series of autopsy cases who had died outside the hospital and represented a comprehensive sample of the general population. Current legislation in Finland for determining the cause of death dates back to 1973. The law on the determination the cause of death (459/1973) states that police has to investigate the cause of death if the underlying cause is not known or deceased had not contacted a doctor or hospital during his/her most recent illness. An investigation was also done when the death was suspected to be unnatural or was unexpected. In these cases, the police requests medico-legal autopsy to be performed. Before 1973 legislation, at the time of collection of the first 1968-1969 series, the basis for performing a medico-legal autopsy was in the old criminal law (39/1889): it was here stated that police had to ask a

doctor to perform an autopsy in cases that remained unclear after external body examination and after reviewing possible medical records of the deceased. In conclusion, there have been no significant changes in legislation that affected the medico-legal autopsy rate or selection during the 50 year period when the autopsy series were collected. The only change has been a steady but slow increase in the number medico-legal autopsies due to e.g. new laws on patient rights and on compensations due to accidents in medical care. At the same time, the rate of clinical in-hospital autopsies has declined due to many reasons such as improved clinical diagnostics and many of these autopsies have also been performed as medico-legal autopsies. Since 1975 (Fig. 1), between 14.7% (1975) and 24.4% (2008) of all deaths in Finland have been subjected to medico-legal autopsy (6). Based on legislation, the medico-legal autopsy rate in Finland thus comprises every fifth of all deaths and includes all out-of-hospital sudden deaths due to coronary heart disease.

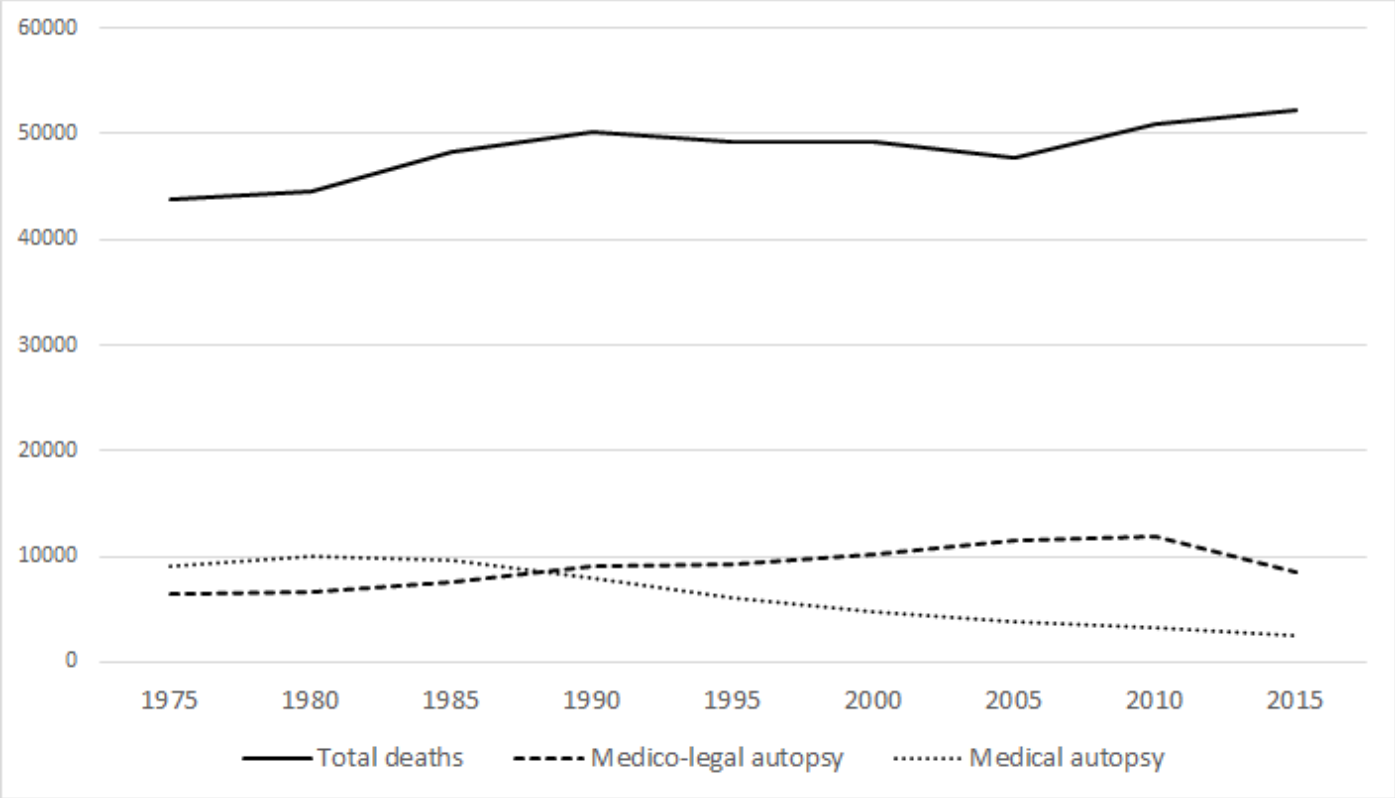

Figure 1 Trends in medico-legal and medical autopsies in Finland 1975-2016 (6).

1. Aortic and Coronary Atherosclerosis in a Finnish Autopsy Series (ACAFAS) 1968-9. This series was collected at Department of Forensic Medicine, University of Helsinki and comprised 569 medico-legal autopsies on Caucasian Finnish men and women, who had died suddenly outside hospital due to diseases or who were victims of violent or other non-natural death in Helsinki capital area and its surroundings (5). Helsinki is the capital of Finland with 0.5 million people and about 10% of the population. The goal of the study was to obtain an unselected cross-sectional sample of the Finnish population and develop an accurate and repeatable methodology to measure the age- and sex-dependent development of atherosclerotic lesions in the Finnish population and compare with findings in International Atherosclerosis Project. The study focused on victims of violent death who were considered to represent the best cross-section of the whole population to study coronary atherosclerosis. ACAFAS study subjects included 433 (76.1%) males and 136 (23.9%) females with the mean age of 47.7 (range 6 - 92 years), which was the lowest of the four series. Children of under age of 5 were excluded from the study as well as cases in which adequate artery specimens were not obtained owing

to crushing, burns, decay etc. No other intentional selection was made (5). ACAFAS series comprised 62.6% of all non-natural deaths that were autopsied at the Department of Forensic Medicine during the time of collecting the study series.

2. Helsinki Sudden Death Study (HSDS) 1981-2 and 1991-2. This autopsy series was collected in the Helsinki city area and its surroundings in two phases with ten years interval. It comprises altogether 700 male autopsies performed at the Department of Forensic Medicine, University of Helsinki. The first HSDS study was launched in 1981 as a parallel independent study to the national WHO MONICA project in order to focus on the epidemiology and risk factors of sudden prehospital cardiac death (SCD) and to follow changes in its epidemiology. The first autopsy series was collected in 1981-1982 (400 cases) and the second one ten years later in 1991-1992 (300 cases). All subjects were Caucasian Finnish Men with the mean age of 53.5 years (range 35-69 years), who died out of the hospital. Decomposed or mutilated bodies were excluded from the series, as above. Because HSDS series was intended to study the epidemiology of SCD, the representativeness of the series was examined using data on all deaths in the area during the study period obtained from the Statistics Finland. It turned out that HSDS study male deaths covered 25.3 % of all sudden out-of-hospital deaths among 35-69-year-old males during the study period in Helsinki. The series also covered 35.0% of all death due to ischemic heart disease (IHD), including 21.7% of all MI deaths and all prehospital deaths due to first MI, as well as 50.9% of all violent deaths (accident, suicide, or homicide) in this area.

3. Tampere Coronary Study (TCS) 2001-4 comprised 746 Caucasian Finnish autopsy cases who had died suddenly out-of-hospital between in Tampere city area and subjected to autopsy at the Department of Forensic Medicine, University of Tampere. Tampere is the third largest city in Finland with 200 000 inhabitants situating 150 km north from Helsinki. The focus in TCS was to study the genetics of CHD by making use of the exact phenotype of coronary artery disease that was only possible to obtain by direct morphometric measurements of autopsy coronaries. DNA was extracted from postmortem blood for genome-wide analysis (GWA). Of the cases, 482 (64.6%) were men and 264 (35.4%) were females. Subjects included all ages with a mean age of 62.0 years (range 0-97 years).

4. Tampere Sudden Death Study (TSDS) 2010-15 was collected in Tampere and consists of 700 autopsy cases of both men and women. This series focused on collecting more cases for GWA studies as well as fresh coronary samples for molecular microbiology and immunohistochemistry to study the genetics of SCD as well as the inflammation hypothesis of coronary artery disease. One of the goals was also to collect gut microbiome for molecular microbiological analyses to study the association between microbiome and CHD. Of the cases 515 (73.6%) were men and 185 (26.4 %) females. Subjects included all ages above 15 years. The mean age in the series was 63.5 years (range 16-95 years).

Table 1 Summary of cohort properties of the study series.

	ACAFAS	HSDS (Series 1)	HSDS (Series 2)	TCS	TSDS
N	569	400	300	746	700
Study period	1968-1969	1981-1982	1991-1992	2001-2004	2010-2015
Subjects					
Age (years)	47.7 ± 15.9	53.8 ± 9.5	52.1 ± 9.6	62.0 ± 19.6	63.5 ± 16.3
Age range (years)	6 - 92	35 - 69	35 - 69	0 - 97	16 - 95
Sex (males%)	76.1	100	100	64.6	73.6
Height (cm)	168.1 ± 9.2	175.5 ± 6.9	173.4 ± 7.1	168.6 ± 14.1	171.3 ± 9.4
Weight (kg)	68.3 ± 14.2	74.9 ± 16.3	75.8 ± 16.8	78.6 ± 21.4	84.1 ± 21.8
Cause of death					
Cardiac related (%)	30.4	42.8	39.0	27.2	48.6
- CHD (%)	-	37.5	26.7	21.5	30.0
- Other cardiac causes (%)	-	5.3	12.3	5.7	18.5
Other disease (%)	15.5	19.3	21.0	32.3	22.9
Non-natural (%)	54.1	38.0	40.0	40.5	28.5
Risk factors					
BMI	24.0 ± 4.2	24.2 ± 4.5	25.1 ± 5.0	27.3 ± 6.1	28.5 ± 6.6
Smoking (%)	-	84.5	78.2	-	53.3
Hypertension (%)	-	14.3	16.7	-	28.0
Diabetes (%)	-	27.4	14.3	-	26.7
Risk factor interview success ratio (%)	-	83.8	41.3	-	64.1

What has been measured?

Basic parameters. All autopsies performed in these study series were complete medico-legal autopsies and included routine forensic toxicological and microscopic analyzes in addition to research samples. Basic parameters in all studies include age, sex, height and weight of the deceased, as well as cause and manner of death. The mean age increases during the study period and also differs slightly between the series due to different inclusion criteria. As there is a known inverse age-cohort effect on height with older subjects being shorter, there is no trend in height visible between the study series due to differences in inclusion criteria. In contrast, the mean weight, as well as the BMI, shows a constant increase over time, which is consistent with the knowledge of increased obesity seen in many Western countries. In HSDS and TSDS other traditional CHD risk factors such as smoking, the presence of hypertension or diabetes were obtained by interviewing relatives or another person, who knew the subject closely using a structured questionnaire. The proportion of those who smoked showed a decrease and the percentage of reported hypertension increased, but the inclusion criteria for HSDS and TSDS are so different that any conclusions cannot be drawn.

Cause of Death. In every case, underlying and immediate causes of death were determined according to the rules of WHO and International Classification of Diseases (versions 8-10). The rate of cardiac-related deaths varied between 48.6% and 27.2%, depending partly on differences in the age inclusion criteria. In ACAFAS series the percentage of

non-natural deaths was the highest (54.1%). This was due to the lowest rate of medico-legal autopsies performed on natural death cases at that time when more autopsies in natural death cases were performed by clinical pathologists in hospitals.

Morphometric measurements of atherosclerosis. In all studies, the extent of atherosclerotic lesions of the coronary arteries and other artery samples such as the aorta or carotid arteries were measured precisely using morphometric methods available at the time of the study (Fig. 2). In HSDS, TCS and TSDS the severity of cerebral atherosclerosis was scored visually. In ACAFAS a point-counting method using a transparent plastic grid was used to measure coronary and aortic atherosclerosis from formalin-fixed arterial samples attach on cardboards (Fig. 2, A). In HSDS series, surface areas of atherosclerotic lesions of formalin-fixed coronary artery specimens attached on cardboards were measured using computer-assisted morphometry directly on artery segments. In TCS a 2 cm piece of the proximal “diseased” part of LAD and a similar control sample from the most “healthy” site of the same artery was fixed in formalin for 24 h, processed through raising saccharose series and stored in +4°C in the presence of 0.1% of sodium azide to prevent bacterial growth (Fig. 2, E). In TSDS the pericardium was opened aseptically using $NaOH/H_2O$ /EtOH treated instruments and sterile gloves. The heart was put on sterile surgical covering and coronary arteries were dissected free from the surface of the heart using sterile instruments and stored on sterile Petri dishes on ice under cover until transferred at the same day to the laboratory. In the laboratory, they were washed with 2 ml sterile phosphate buffer and photographed using a microscope camera and Olympus Cell D software. In addition, transverse sections from the most severe coronary plaque/ complicated ulcerated plaque/ thrombus site from the left anterior coronary artery (LAD) and the right coronary artery (RCA) were taken for histology under sterile conditions and with sterile instruments. Morphometric measurement in TCS and TSDS were performed in photographs of the arteries *in silico* using Olympus Cell D software (Fig. 2, E).

Coronary stenosis. Coronary stenosis percentage was estimated visually in ACAFAS, whereas in HSDS stenosis was measured in silicone rubber casts (Fig. 2, B-C) of the coronaries (7). In TCS and TSDS coronary stenosis was measured *in silico* from histological HE-stained sections using an Olympus BX-51 microscope equipped with a camera and Olympus Cell D software (Fig. 2, E-H).

Definitions of atherosclerotic lesion types. In ACAFAS and HSDS series arterial specimens were stained using Sudan IV and lesion types classified according to the instructions presented by WHO study group (5,8,9) into following categories: fatty streaks, raised lesion, complicated lesion, fibrous plaque and calcification. In TCS and TSDS coronaries were classified as above but were also categorized into Stary classes (I-VI), nowadays called American Heart Association (AHA) types (I-VI) using photographs and microscopical slides (Table 2).

Genetic association studies. In HSDS first series in 1981-2, DNA was extracted from 15-year-old paraffin blocks of the myocardium in 1996 using the method of Isola et al. (10). The quality of DNA was suitable only for studying single nucleotide polymorphisms (SNP) such as APOE (9). DNA validity was good enough for genome-wide analysis (GWA) in HSDS second series in 1991-2 where DNA was extracted from frozen heart muscle using the standard phenol-chloroform method. In TCS and TSDS DNA was extracted from postmortem blood using a Qiagen DNA extraction kit. GWAS analyses from HSDS series were performed with Affymetrix 6.0, in TCS using Metabochip TM and TSDS using Exomecorechip TM.

Molecular microbiological samples from artery segments. In TCS, DNA extraction was performed from -20°C stored formalin fixed coronary segments followed by real-time quantitative PCR (qPCR) using *in-house* primers to detect the amount of total bacterial DNA as well as the presence and amount of 14 different bacterial genomes that have been implicated in the pathogenesis of CHD (Fig 2, E) (11). In TSDS, DNA extraction was performed from fresh "sterile" coronary segments immediately after the autopsy. The sterility was obtained by handling the heart using instruments soaked in NaOH/EtOH/sterile water. These DNA samples from atherosclerotic plaques were subjected to qPCR as above. In 100 cases, next-generation sequencing (NGS) was performed to study the microbiome within coronary artery plaques.

Gut microbiome. Fecal samples of autopsy cases were frozen and kept in -80°C until bacterial DNA was extracted using a commercial DNA extraction kit (Zymo Fecal DNA Kit (Zymo Research Corporation, California, USA). The total amount of bacteria, as well as ratios of major intestinal bacterial communities (Bacteroides spp., Clostridium leptum group, Clostridium coccoides group, Bifidobacterium spp., Enterobacteriaceae, Lactobacillus spp.) and Streptococcus spp, were measured in feces and coronary plaques of the same male autopsy using qPCR (12).

Interview on the CHD risk factor of the next-of-kin. In HSDS detailed information on traditional CHD risk factors was collected by personal interviews of the relatives using a questionnaire. In TSDS the questionnaire was mailed to the widow, children, closest relative or in some cases to a close friend of the deceased. If there were no answer, second and third mailing rounds were applied. The questionnaire comprised a set of 50 questions concerning previous diseases and possible risk factors of SCD such as smoking, hypertension or diabetes. The drinking habits of the deceased were ascertained by a validated 14-item, quantity-frequency questionnaire incorporating questions from the Alcohol Use Disorders Identification Test (AUDIT) about drinking quantity, quality of the beverage, drinking frequency and weekend drinking or binge drinking of the deceased's last year. In HSDS an informant could be contacted and interview completed in altogether 71.4 % cases whereas in TSDS the interview using a mailed questionnaire succeeded in 61.5% of cases.

Other samples and parameters. In HSDS 1991-2, a routine dental panoramic x-ray (Fig. 2, D) was taken in the autopsy room (13) whereas in TSDS the number of teeth was calculated, but there was no possibility to take a dental x-ray. In TCS and TSDS also neuropathological samples were taken to study the association between atherosclerosis and dementia (14). In TSDS, we also took fecal samples aseptically from the rectum for DNA extraction and to study the association between coronary atherosclerosis and gut microbiome as determined by bacterial genetic techniques (12).

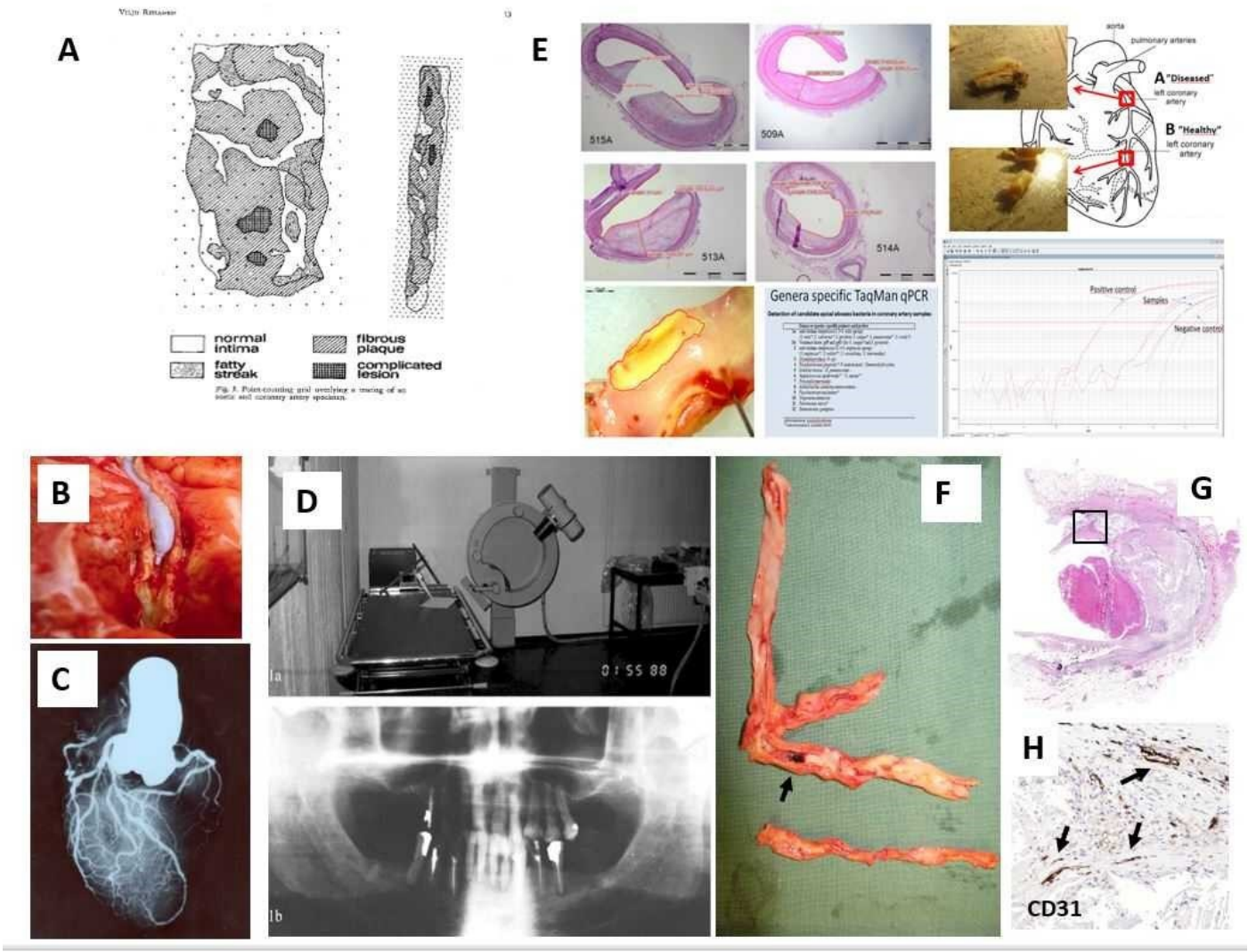

Figure 2 Measurement of aortic and coronary atherosclerotic lesions by a point-counting method in ACAFAS using a transparent plastic grid (A). Coronary artery stenosis in both HSDS series was measured in silicone rubber casts made radio-opaque with lead oxide (B). Panoramic dental radiography of SCD victims in HSDS 2 series was performed on a transportable table located in the autopsy facilities using a panoramic tomography apparatus (Zonarc, Palomex Oy, Finland) placed in a horizontal position on the wall (C and D). Illustrative schema on sampling proximal and distal coronary segments in TCS and measuring coronary artery atherosclerosis and stenosis (E). In TSDS, the focus was to obtain fresh non-ruptured and especially ruptured and thrombosed (arrow) coronary plaques (F) for immunohistochemical studies on the ruptured atheroma (G). Arrows show neovasculature (CD31) inside atheroma (H).

Table 2 Summary of collected data in each study

	ACAFAS	HSDS (Series 1)	HSDS (Series 2)	TCS	TSDS
Study period	1968-1969	1981-1982	1991-1992	2001-2004	2010-2015
Sex	X	X	X	X	X
Height and weight	X	X	X	X	X
BMI	X	X	X	X	X
Interview of relatives (CHD risk factors)	-	X	X	-	X
Cause of death	X	X	X	X	X
Heart weight	X	X	X	X	X
Heart dimensions	X	X	X	X	X
Coronary artery stenosis%	X	X	X	X	X
Coronary artery atherosclerosis lesions surface%	X	X	X	X	X
Abdominal aorta atherosclerosis lesions surface%	X	X	X	-	X
AHA (Stary) classification of coronary plaques	-	-	-	X	X
Thoracic aorta atherosclerosis lesions surface%	X	-	-	-	-
Carotis artery atherosclerosis lesions surface%	-	-	-	-	X
Cerebral artery atherosclerosis score	-	X	X	-	X
Coronary artery histology and immunohistology	-	-	-	X	X
Abdominal aorta histology	-	-	-	-	X
Heart muscle histology	-	X	X	X	X
Heart muscle immunohistology	-	-	-	X	X
DNA extraction	-	X	X	X	X
Genome Wide Analysis (GWA)	-	-	X	X	X
Coronary bacterial genetics (qPCR)	-	-	-	X	X
Blood and urine samples	-	-	-	X	X
Dental pathology	-	-	X	-	X
Gut microbiome	-	-	-	-	X
Neuropathology	-	-	-	X	X

What has it found? Key findings and publications

Table 3 Summary of key findings

ACAFAS Aortic and Coronary Atherosclerosis in a Finnish Autopsy Series	➢ Fatty streaks in LAD were demonstrated already in children below 10 years old whereas first raised lesions were discovered in males aged 15-24 years, complicated lesions in males aged 35-44 years. However, coronary calcification was already seen in males already in the age group 25-34 years. (5) ➢ Coronary narrowing by 50% was observed both in males and females first in the age group of 35-44 years. In males, this was the same age as reported in USA soldiers killed in Vietnam. (5) ➢ Despite Finland showing one of the world's highest CHD mortality, the severity of coronary and aortic atherosclerosis in the Finnish autopsy series measured as areas of raised atherosclerotic lesions (15%) was similar than among USA whites (18.3%), USA blacks (14.5%) and Norwegians (17.8%) of the International Atherosclerosis Project. (5)

HSDS Helsinki Sudden Death Study	➢ Men < 53 years old with the APOE e4/3 genotype showed 61% larger total atherosclerotic lesion area in the RCA and 26% larger area in the LAD than did men with the e3/3. (9) ➢ HPA-2 Met/VNTR B haplotype of the platelet von Willebrand factor and thrombin receptor protein GP Ib-V-IX may be considered to be a major risk factor of coronary thrombosis, fatal MI, and SCD in early middle age. (15) ➢ The effects of alcohol on the heart in middle-aged men are dose dependent but partly non-linear. In the absence of coronary artery disease, LV size shows a U shaped reduction with increasing daily alcohol use accompanied by an increase in RV size with very heavy drinking. (16) ➢ Poor oral health was associated (p = 0.053) with the risk of sudden cardiac death along with age, smoking, and body mass index. This association was especially strong (p = 0.009) among victims < 50 yrs. (13) ➢ Middle-aged white men carrying the DD genotype of the alpha(2B)-AR have a significantly increased risk for SCD and AMI, especially before the age of 55 years. (17)
TCS Tampere Coronary Study	➢ CRP immunoreactivity is associated with the progression of atherosclerosis, and especially with unstable coronary plaques. The immunoreactivity could cease at the stable calcified stages of atherosclerosis. (18) ➢ A GWAS meta-analysis of 185 thousand CAD cases and controls, interrogating 6.7 million common (MAF>0.05) as well as 2.7 million low frequency (0.005<MAF<0.05) variants confirmed most known CAD loci, identified 10 novel loci, eight additive and two recessive loci, that contain candidate genes that newly implicate biological processes in vessel walls. (19) ➢ Genetic variation in proprotein convertase subtilisin/kexin type 9 (PCSK9) gene is associated with the risk of large-vessel atherosclerosis stroke subtype and suggest that the risk is mediated by the severity of intracranial atherosclerosis. (20) ➢ Hypertension interacts with IL-18 gene promoter 2137 G/C polymorphism, affecting the risk of SCD and the development of coronary atherosclerosis. (21)
TSDS Tampere Sudden Death Study	➢ A weighted genetic risk score (GRS_{CAD}) was formed from variants most strongly associating with CAD identified by the CARDIoGRAMplusC4D Consortium explaining 10.6% of the heritability of CAD [153 single-nucleotide polymorphisms with r2 < 0.2] predicted significantly the risk of SCD due to coronary artery disease. (22) ➢ DNA of *Clostridium leptum* group and of pathogenic *Enterobacteriaceae* increase in gut microbiome with age and can be detected in the same person's coronary plaques along with pathogenic *Streptococcus* spp. associating with more severe coronary atherosclerosis. (12)

What are the main strengths and weaknesses?

Due to the Finnish legislature on determining causes of death, the coverage of medico-legal autopsies is high, comprising in average 20% of all the deaths during the study period. Including all violent deaths as well as sudden unexpected cardiac deaths occurring out-of-hospital, it enables an exceptional possibility to study the epidemiology of sudden cardiac death by non-natural deaths and deaths due to non-cardiac diseases serving as controls. The present set of autopsy series is unique because studies presented in this cohort profile are based on detailed plaque measurements and classification. The scope of the studies has changed during 50 years from basic studies on the presence and significance of atherosclerosis in different age groups in males and females groups as well as international comparisons to genetic and molecular microbiological studies. Most recently, the possible role of gut microbiome has been brought into attention (12).

Weaknesses of these autopsy series include that due to the nature of the series comprising out-of-hospital deaths we do not have cholesterol, blood pressure or glucose measurements in our series. We have partly overcome this problem by interviewing the next-of-kin of the deceased on these risk factors, but this kind of data most probably underestimates the true prevalence of CHD risk factors.

Despite the beneficial trends in CHD mortality and declining rate of coronary atherosclerosis observed in industrialized countries such as the USA since 1960 (23), cardiovascular diseases remain one of the most significant and fatal diseases worldwide causing excess mortality and significant burden to national health care services. Although research has confirmed a key role for cholesterol in the pathogenesis of coronary heart disease (CHD) (24), it is still not known why a cholesterol-containing coronary atheroma ruptures causing myocardial infarction. One of the main obstacles has been that atherosclerotic lesions in the commonly used genetically modified mice seldom develop plaque disruption with thrombosis. Therefore, autopsy studies are still needed as they are the best way to provide human samples for the research to gain insight into the remaining mysteries of coronary heart disease.

Can I get hold of the data? Where can I find out more?

We welcome all proposals for collaboration concerning series with GWA data (HSDS, TCS, TSDS) as well as with coronary histology (TCS and TSDS). Inquiries for further information about the studies, requests for the data and other questions should be directed to Prof. Pekka Karhunen (pekka.karhunen@uta.fi) and in questions concerning collaboration in genetic studies to Prof. Terho Lehtimäki (terho.lehtimaki@uta.fi).

Profile in a nutshell

- Four independent autopsy studies collected in 1968 - 2015 at the University of Helsinki and the University of Tampere in Finland to study epidemiology, pathology, risk factors and genetics of atherosclerosis and sudden cardiac death comprising a total of 2715 autopsy cases who died out-of-hospital due to cardiac diseases, non-cardiac diseases or non-natural reasons.
- Studies included are the cohort are Aortic and Coronary Atherosclerosis in a Finnish Autopsy Series (1968-9), Helsinki Sudden Death Study (1981-2 and 1991-2), Tampere Coronary Study (2001-4) and Tampere Sudden Death Study (2010-5).

- All studies are based on coronary measurements at the vessel wall level and include morphometric measurements of atherosclerosis, coronary stenosis and lesion types.
- Depending on the study, the dataset also includes genetic studies, risk factor interviews and microbiological and other samples.
- Request for data and other inquiries should be directed to Prof. Pekka Karhunen (pekka.karhunen@uta.fi) and questions concerning genetic studies to Prof. Terho Lehtimäki (terho.lehtimaki@uta.fi)

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