

Assessment of subclinical atherosclerosis and its risk
factors at population level

PhD Thesis

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1. INTRODUCTION

In Hungary, almost half of the mortality is due to cardiovascular disease, atherosclerosis is the cause in 42%. While mortality and morbidity rates have decreased in Western-Europe, Hungary falls behind in that regard.

1.1 Atherosclerosis

Atherosclerosis is the origin of a notable proportion of cardiovascular disease. Therefore, it has an important role in prevention. Atherosclerosis is an alteration of the intimal layer of arteries that is characterised by focal deposition of lipids, connective tissue and blood components and is associated with fibrous histogenesis, calcification and injury of media. Its important steps are the following: Foam cell formation, development of fatty streak, fibrous plaque, calcification and complex lesion.

Endothelial dysfunction has a significant role in the development of atherosclerosis. In case of endothelial dysfunction, the synthesis, bioavailability and effectiveness of nitrogen monoxide decreases, and its elimination increases. The surface of the endothelia loses its anticoagulant coating, its barrier function becomes damaged, its adhesivity towards white blood cells and platelets increases.

Plaque rupture and atherothrombosis can occur as a complication of atherosclerosis. It has several clinical manifestations such as myocardial infarction, instable angina, transient ischemic attack and stroke.

Risk factors of atherosclerosis were first described in the Framingham study. Factors that are not preventable include gender, age and genetics. Factors that can be influenced are smoking, hypertension, hyperlipidemia, obesity, diabetes mellitus, psychosocial factors and other factors (CRP, uric acid).

1.2 Diabetes mellitus

Type 2 diabetes mellitus (T2DM) is one of the most important cardiovascular risk factors. Prevalence of diabetes is around 8% worldwide, prevalence of prediabetes is 20-35%. 70-80% of patients with T2DM die as a result of cardiovascular disease. At the time of diagnosis, complications may already be present (macrovascular in 30% and microvascular in 12%).

These data imply that asymptomatic patients at risk for diabetes, with elevated Finnish Diabetes Risk Score (Findrisk score, FR) also have higher risk for atherosclerosis.

Findrisk questionnaire is used for the estimation of 10-year risk of developing diabetes. It is calculated from 8 parameters: age, body mass index, waist circumference, physical activity, consumption of vegetables and fruits, antihypertensive treatment, elevated blood glucose level and family history. Risk categories according to the score are the following. A score below 7 indicates a very low risk. A score of 7-11 indicates a slightly elevated risk as 4% of these patients develop diabetes. The risk is moderately elevated (15%) if the score is 12-14, high (33%) if the score is 15-20 and very high (50%) if the score is above 20.

1.3 Uric acid

Uric acid is the product of purine catabolism by xanthine-oxidase. Part of purine is endogenous, other part is from exogenic intake. Uric acid is excreted in the urine and faeces. Serum level of uric acid depends on the balance of intake, synthesis and excretion. An upset balance may lead to hyperuricemia. Normal level of uric acid is higher in men (416 $\mu\text{mol/l}$) than women (375 $\mu\text{mol/l}$). Prevalence of hyperuricemia is 5-20%.

Elevated serum level of uric is associated with several cardiovascular risk factors and diseases, such as hypertension, diabetes mellitus and metabolic syndrome. In a recent study, higher uric acid levels were associated with an increased risk of hospitalization and mortality related to cardiovascular disease. Its role as a risk factor for cardiovascular disease is studied extensively; however, whether the association is independent of conventional risk factors is controversial.

1.4. Relevance of subclinical atherosclerosis

Prevention of cardiovascular disease is all-important at both individual and society levels. Resources in healthcare are limited, especially those for preventive purposes, therefore it is crucial to select those who benefit from screening, follow-up or therapy. Examining subclinical atherosclerosis may be useful for decision-aiding. Measurement of ankle brachial index, carotid plaques, carotid intima-media thickness (CIMT) and coronary calcification are methods for detecting subclinical atherosclerosis.

The layers of the arterial wall appear as a double-line pattern on carotid duplex scans. Intima-media thickness is associated with atherosclerosis seen on histological samples. Therefore, IMT could represent the state of the whole vascular system. A value above 0.9 mm is considered pathologic. It seems that its association with cardiovascular disease is graded but not linear. According to the 2012 ESC guidelines, it should be considered (IIa) as a complementary method in risk assessment, but in the 2016 guideline it is not recommended (III).

Coronary calcification (CAC) can be measured on native, prospectively ECG-triggered CT scans performed during

breath-hold. Quantity of calcification is expressed by Agatston score that is the arithmetical product of calcification area and a density coefficient. CAC is commensurate to the total plaque burden. Negative predictive value of a 0 value is outstandingly high. Several studies showed the association of CAC with cardiovascular events. A score above 300 or the 75th percentiles associated with high risk. According to the 2016 ESC guidelines its use may be considered (IIb) if the SCORE is around the 5% or 10% thresholds, but its routine use is not recommended.

2. OBJECTIVES

The aim of my work was to evaluate the cardiovascular risk profile of a central-Hungarian population and to analyse the association of CIMT with prediabetes and CAC with uric acid in the asymptomatic population of Budakalász Study.

My objectives were

- 1) to assess the prevalence of cardiovascular risk factors, hidden morbidity and health indicators.
- 2) to analyse the association of carotid intima-media thickening and elevated HbA1c% level, Findrisk score and conventional risk factors.
- 3) to analyse the association of the presence of coronary calcification and serum uric acid level and conventional risk factors.
- 4) to analyse the association of the severe coronary calcification and serum uric acid level and conventional risk factors.

3. METHODS

3.1. Subjects

A cross-sectional voluntary cardiovascular screening program (Budakalász Health Survey) was performed, in 2011–2013. The target population was the adult population of the selected central Hungarian town; participation rate was 30.3% (2420 participants). The collected data and samples were stored in the Városmajor Biobank of Semmelweis University.

3.2 Questionnaire, physical examination and laboratory tests

The first part of the screening program is the Health Interview Survey. It is based on the European Health Interview Survey (EHIS) with added questions on cardiovascular diseases. Questions cover the following topics: socioeconomic status (e.g. degree, income, marital status); health status, health determinants; lifestyle (alcohol consumption, sport activities, smoking habits); psychological factors, environmental factors. Then, medical history was acquired, anthropometric measurements were taken (BMI, waist circumference), 12-lead ECG was recorded, echocardiography and carotid duplex scan were performed. Blood pressure was measured on both arms and legs after resting in a supine position, systolic value below 140 Hgmm and diastolic value below 90 Hgmm were considered as normal on the arms. Venous blood samples were taken and laboratory tests were performed at the Heart and Vascular Center (blood and urine test panel: blood cell count, renal function, hepatic enzymes, electrolytes, albumin, glucose, LDL-, HDL-cholesterol, TG, uric acid, hsCRP and HbA1c%). Concentration of SUA was measured by using a colorimetric assay with rigorous quality control. HbA1c level measurement was done on all participants from haemolysed whole blood using a turbidimetric inhibition immunoassay (Tina-quant HbA1c). HbA1c is reported in mmol/mol and

HbA1c% by the device. Prediabetes was indicated by an HbA1c% level of 5.7%–6.4%, if HbA1c% level was equal to or above 6.5%, it was considered as screen-detected diabetes.

3.3 Carotid intima media thickness measurements

CIMT measurement was performed following the instructions of the 2012 Mannheim Consensus. Intima- media thickness was measured within a region free of plaque with a clearly identified double-line pattern. Carotid duplex scans of 1475 participants were evaluated offline and semi automatically by GE EchoPac software (General Electric Healthcare, Chicago, Illinois, USA). Measurement was performed on the common carotid artery on both sides 1 cm proximal from the bifurcation with 200 (± 10) measurement points. Maximum, minimum and mean values were recorded. The average values of left and right sides were used for analysis in cases of both mean CIMT and maximal CIMT. CIMT was considered normal under 0.9 mm.

3.4. Heart CT scan

CAC scan (Brilliance iCT, Philips Healthcare, Best, The Netherlands) was performed in men older than 35 years and women older than 40 years on a voluntary base. Radiation dose was 0.5 mSv or less. Oral beta blockers were administered, if the heart rate was above 65 bpm. Prospectively, ECG-triggered scans were acquired. Quantitative analysis of coronary calcification on the axial images was performed using a commercially available software application (Calcium scoring, Heartbeat-CS, Philips Healthcare). All coronary artery plaques with an area of ≥ 1 mm² and a density of greater than 130 Hounsfield Units (HU) were identified by the software, and then real coronary plaques were selected manually by an expert observer which allow the semi automatic software to calculate the Agatston score,

calcification area and volume. We used 0 and 300 as cut-off values in the analyses.

3.5 Study groups

Participants with a history of cardiovascular diseases (myocardial infarction, angina pectoris, percutaneous coronary intervention, coronary artery bypass graft, heart failure, cardiomyopathy, stroke, transient ischemic attack, peripheral artery disease) were excluded from the analysis of subclinical atherosclerosis. Also, those with manifest diabetes or kidney failure were excluded from the analysis of prediabetes and those on regular allopurinol therapy were excluded from the analysis of uric acid.

For the purpose of analysing the association between HbA1c levels, Findrisk score and CIMT, participants were categorized into four groups: *control* (HbA1c% < 5,7%, FR < 12), *HbA1c only* (HbA1c% ≥ 5,7%, FR < 12), *Findrisk only* (HbA1c% < 5,7%, FR ≥ 12) and *combined* (HbA1c% ≥ 5,7%, FR ≥ 12).

In the coronary calcification-uric acid study based on the SUA levels participants were stratified into terciles by gender.

3.6. Statistical methods

Microsoft Excel and PASW Statistics 18 were used for statistical analysis. All continuous variables were expressed as mean with standard deviation (SD) or as median with interquartile range as appropriate depending on the distribution of the values, whereas categorical variables were expressed as percentage. Comparisons of means, medians, and proportions were performed with variance analysis, Kruskal-Wallis test, Jonckheere-Terpstra test, chi-square tests, and Cochran-Armitage test respectively. Spearman correlation was used to

test the association between SUA and CAC. Multivariate regression analysis and power analysis were performed. All analyses were performed two-tailed and $p < 0.05$ was considered significant.

4. RESULTS

Mean age of participants in the Budakalász Study was 54.8 \pm 14.8 years, proportion of men was 41.2%. Subjective health was rated good or very good in 56.8%. BMI was above 25 in 70.3% of participants (females: 64%, males:79.4%) and was above 30 in 31.7%. 12.9% of obese participants considered themselves normal weight. Hypertension in medical history was present in 47.7%, 89.2% of the cases were treated. We have found elevated blood pressure in 45.5% generally and in 63.5% of treated cases. According to the questionnaire 38.2% of those with untreated hypertension and 6.5% of those with treated hypertension did not acknowledge their disease. Hyperlipidaemia in medical history was present in 27.3%, 50.8% of the cases were treated medically. We have found elevated LDL-cholesterol level in 44.1% generally and in 25.6% of treated cases. Women had a more favourable lipid profile. At the time of the screening 15.2% of participants were active smokers.

4.2. Carotid intima-media thickness and prediabetes study

The mean Findrisk score of the 1475 participants was 8.7 ± 4.6 (males: 8.9 ± 4.5 , females: 8.3 ± 4.6 , $p = 0.007$). Findrisk was 12 or above in 27.3%. The mean HbA1c% level was $5.6\% \pm 0.5$. Elevated HbA1c level was found in 39.5% (34.7% of males, 42.1% of females, $p = 0.014$). HbA1c% level increased

significantly in each Findrisk category ($p < 0.001$), and the incidence of prediabetes (HbA1c% 5.7-6.4) and “recent” diabetes (≥ 6.5) was significantly higher in the higher FR risk groups. Screen-detected diabetes mellitus was found in 52 cases (3.6%).

Mean age was significantly higher in groups with poorer glycaemia status. The proportion of men was highest in the *control* group and lowest in the *combined* group. The proportion of former or current smokers did not differ significantly in the groups. The prevalence of hyperlipidemia and hypertension and mean BMI showed an increasing trend across the groups. As expected, mean HDL cholesterol level was lowest in the *combined* group and highest in the *control* group. The characteristics of the participants are shown in Table 1.

Table 1 – Patient characteristics in the four groups

Risk factors, n (%) / mean (SD)	control 711 (48.2%)	HbA1c 360 (24.4%)	Findrisk 190 (12.8%)	combined 214 (14.5%)	p
Gender (male)	316 (44.4%)	135 (37.5%)	75 (39.3%)	75 (35.0%)	0.033
Age (years)	45.5 (13.6)	55.8 (13.2)	57.5 (11.7)	62.1 (9.3)	<0.001
Smoking	288 (40.6%)	134 (37.2%)	74 (38.9%)	92 (43.0%)	0.546
Elevated blood pressure	191 (26.9%)	146 (40.6%)	82 (43.2%)	104 (48.6%)	<0.001
Hyperlipidaemia	145 (20.4%)	118 (32.8%)	68 (35.8%)	92 (43.0%)	<0.001
BMI. kg/m²	25.1 (3.8)	27.3 (4.4)	30.6 (4.8)	32.8 (5.1)	<0.001
Systolic blood pressure (Hgmm)	130 (17.3)	138.3 (18.8)	137.5 (16.1)	141.8 (17.3)	<0.001
HDL-cholesterol (mmol/L)	1.55 (0.46)	1.50 (0.47)	1.41 (0.39)	1.40 (0.45)	<0.001
LDL-cholesterol (mmol/L)	3.22 (0.9)	3.58 (1.07)	3.45 (0.87)	3.46 (0.93)	<0.001
HbA1c%	5.31 (0.23)	6.0 (0.51)	5.35 (0.25)	6.06 (0.51)	<0.001
Findrisk	6.0 (3.2)	7.8 (2.9)	14.2 (2.4)	14.4 (2.6)	<0.001

Among the four groups based on HbA1c% levels and FR, mean CIMT mean and CIMT max showed an increasing tendency ($p < 0.001$). Also, the frequency of pathological mean and maximal CIMT showed significant difference with a higher frequency in groups with higher risk.

Both Hba1c and Findrisk score showed a significant positive correlation with maximum CIMT ($r = 0.233$, $p < 0.001$, $r = 0.469$, $p < 0.001$; respectively) and mean CIMT ($r = 0.239$, $p < 0.001$, $r = 0.454$, $p < 0.001$; respectively).

Multivariate logistic regression analysis conducted in a model adjusted to conventional risk factors that are not part of the FR scoring system (such as sex, smoking status, high blood pressure and history of treated or not treated hyperlipidaemia) revealed that both elevated HbA1c% and Findrisk score above 12 were independent predictors of pathological CIMT.

4.3. Coronary calcification- uric acid study

Results of 281 participants were analysed. Age, BMI, hypertension, HDL, triglyceride, and creatinine levels were significantly different in the three tercile groups. In the 3rd SUA tercile group, age, BMI, and creatinine level were higher, whereas HDL was lower compared to the other groups. Also, the frequency of hypertension increased gradually in the groups and was highest in the third. Triglyceride level and SBP were lowest in group 1, and similarly high in groups 2 and 3. There was no significant difference in the gender distribution among the three groups. Clinical baseline characteristics of the 281 patients are described in Table 2.

Table 2- Patient characteristics in the three groups

Risk factors n(%), mean (SD)	1. SUA tercile (n=93)	2. SUA tercile (n=94)	3. SUA tercile (n=94)	p
Age	57.22 (11.67)	60.54 (11.21)	62.30 (9.10)	0.005
Gender (male)	38 (40.9%)	39 (41.5%)	39 (41.5%)	0.995
BMI	25.81 (3.92)	28.23 (5.19)	29.55 (4.96)	<0.001
Hypertension	32 (34.4%)	47 (50.0%)	63 (67.0%)	<0.001
Hyperlipidaemia	36 (38.7%)	35 (37.2%)	42 (42.7%)	0.545
Diabetes mellitus	7 (7.5%)	13 (13.8%)	8 (8.5%)	0.301
Smoking	17 (18.3%)	10 (11.6%)	7 (7.4%)	0.066
Systolic blood pressure	132.8 (16)	138.9 (18)	137.1 (17.0)	0.046
Diastolic blood pressure	79.3 (9.1)	81 (8.9)	80.6 (9.9)	0.214
LDL-cholesterol	3.39 (1.00)	3.54 (0.98)	3.62 (1.01)	0.269
Cholesterol	1.65 (0.49)	1.53 (0.49)	1.49 (0.46)	0.049
Triglyceride	1.97 (1.31)	2.49 (1.56)	2.48 (1.39)	0.019
Total cholesterol	5.59 (1.14)	5.79 (1.07)	5.84 (1.16)	0.287
Serum creatinine	73.45 (14.01)	74.41 (15.28)	81.50 (17.56)	0.001

Total Agatston score was 0 in 102 cases (36.3%) and above 300 in 39 cases (13.9%). Median total CAC score was 14.34 (IQR 0–107.4). The Jonckheere-Terpstra test for total CAC score confirmed a trend across SUA groups (J-T statistic 13160 ± 724 , $p < 0.001$). Serum uric acid level showed a positive correlation with total coronary calcium score ($r = 0.281$, $p < 0.001$). Frequencies of CAC > 0 , and > 300 was significantly different in the three SUA groups. Also, there was a trend across SUA groups in both cases (CA statistic 9.59, $p = 0.002$ and 8.597, $p = 0.003$, respectively.)

We performed multivariate adjustment for conventional cardiovascular risk factors (gender, age, BMI, hypertension, hyperlipidaemia, diabetes mellitus, smoking status, creatinine, and SUA terciles as covariates). In model 1, the dependent variable was the presence of any coronary calcification (Agatston score > 0), and in model 2, the presence of coronary calcification indicating high CV risk (Agatston score > 300). Multivariate logistic regression analysis revealed that in model 1, SUA was not an independent predictor of the presence of overall (Agatston score > 0) coronary calcification. In model 2, compared to the lowest SUA tercile (reference category), the third SUA tercile is an independent predictor (OR 5.17, $p = 0.010$) for the presence of severe significant coronary calcification (CAC > 300). In this model, male gender (OR 3.041, $p = 0.017$), age (OR 1.13, $p < 0.001$), active smoking (OR 7.152, $p = 0.004$), and history of hypertension (3.336, $p = 0.022$) were independently associated with coronary calcification.

5. CONCLUSIONS

In asymptomatic patients at moderate cardiovascular risk, detecting subclinical atherosclerosis may be a complementary tool in risk assessment. It could aid the therapeutic decision and draw attention to the already present vascular lesions

that may improve patients' compliance. In our study, prediabetic state, elevated diabetes risk and serum uric acid level showed an association with the presence of subclinical atherosclerosis independent of other factors.

1) The prevalence of cardiovascular risk factors is notable as expected, except for smoking, however the awareness of the population is not satisfactory.

2) Pathologic carotid intima-media thickening was more frequent in those with elevated diabetes risk according to Findrisk questionnaire and HbA1c% level independently of other risk factors.

3) Age, male gender and hypertension but not serum uric acid level were independent predictors of the presence of coronary calcification.

4) There was an independent association of severe coronary calcification and serum uric acid level and conventional risk factors (age, male gender, hypertension and smoking).

6. PUBLICATIONS

List of publications related to the doctoral work

1. **Kiss LZ**, Bagyura Z, Csobay-Novák C, Lux Á, Polgár L, Jermendy Á, Soós P, Szelid Z, Maurovich-Horvat P, Becker D, Merkely B (2019) Serum Uric Acid Is Independently Associated with Coronary Calcification in an Asymptomatic Population. *J Cardiovasc Trans Res* 12(3): 204–210. IF: 2, 756

2. **Kiss LZ**, Bagyura Z, Vadas R, Polgár L, Lux Á, Édes E, Szenczi O, Soós P, Szelid Z, Becker D, Jermendy G, Merkely B. (2017) Signs of subclinical atherosclerosis in asymptomatic

patients at increased risk of type 2 diabetes mellitus. *J Diabetes Complications*, 31: 1293-1298. IF 2,734

3. Bagyura Z, **Kiss L**, Edes E, Lux A, Polgar L, Soos P, Szenczi O, Szelid Z, Vadas R, Jozan P, Bagdy G, Merkely B. (2014) Cardiovascularis szűrőprogram a közép-magyarországi régióban. *Budakalász Vizsgálat. Orv Hetil*, 155: 1344-1352.

Other publications

1. Simon J, Nemeth E, Nemes A, Husveth-Toth M, Radovits T, Foldes G, **Kiss L**, Bagyura Z, Skopal J, Merkely B, Gara E (2019) Circulating Relaxin-1 Level Is a Surrogate Marker of Myocardial Fibrosis in HFrEF. *Front Physiol* 10:2019:690 IF: 3,201

2. Bagyura Z, **Kiss L**, Hirschberg K, Berta B, Széplaki G, Lux A, Szelid Z, Soós P, Merkely B. (2017) Association between VEGF Gene Polymorphisms and In-Stent Restenosis after Coronary Intervention Treated with Bare Metal Stent. *Dis Markers*, 2017:1-7. IF 2,949

3. Bagyura Z, **Kiss L**, Berta B, Szilágyi A, Hirschberg K, Széplaki G, Lux A, Szelid Z, Soós P, Merkely B. (2017) High rate of in-stent restenosis after coronary intervention in carriers of the mutant mannose-binding lectin allele. *BMC Cardiovasc Disord*, 17: 1-4. IF 1,812

4. Szelid Z, Lux A, Kolossvary M, Toth A, Vago H, Lendvai Z, **Kiss L**, Maurovich-Horvat P, Bagyura Z, Merkely B. (2015) Right Ventricular Adaptation Is Associated with the Glu298Asp Variant of the NOS3 Gene in Elite Athletes. *PLoS One*, 10: e0141680. IF 3,057