SUMMARY OF THE PhD THESIS

DETERMINANTS OF SUBCLINICAL ATHEROSCLEROSIS IN OVERWEIGHT PATIENTS

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CONTENTS

Abbreviations ............................................................................................................ iii
GENERAL PART ................................................................. 1
Introduction ............................................................................................................. 1
Chapter 1. Atherosclerosis and cardiovascular risk factors .................................................. 2
Chapter 2. Cardiovascular risk stratification and assessment.................................................. 14
Chapter 3. Subclinical atherosclerosis ................................................................. 19
  3.1. Need for assessment subclinical atherosclerosis............................................. 19
  3.2. Methods for assessment subclinical atherosclerosis ........................................... 21
    3.2.1. Carotid ultrasound ................................................................. 23
    3.2.2. Ankle-brachial index ............................................................. 26
    3.2.3. Coronary calcium score ......................................................... 28
    3.2.4. Ecocardiography ..................................................................... 29
    3.2.5. Arterial stiffness ...................................................................... 31
    3.2.6. Nuclear magnetic resonance .................................................... 33
    3.2.7. Markers of endothelial dysfunction ........................................... 34
    3.2.8. Invasive techniques ................................................................. 36
PERSONAL CONTRIBUTIONS ................................................. 39
Chapter 4. Research motivation ........................................................................... 39
Chapter 5. Material and methods ........................................................................ 41
  5.1. Study design ................................................................................................. 41
  5.2. Ethical considerations ................................................................................. 42
  5.3. Study protocol ............................................................................................... 42
  5.4. Investigations of subclinical atherosclerosis - methodology ............................. 44
    5.4.1. Carotid ultrasound ................................................................. 44
    5.4.2. Ankle-brachial index ............................................................. 46
    5.4.3. Arterial stiffness ...................................................................... 47
Chapter 12. Perspectives open by the PhD research... 122
Chapter 13. General conclusions .............................. 125
BIBLIOGRAPHY ...................................................... 127
ANNEXES ............................................................. 147
  Annex 1. SCORE risk chart ................................. 147
  Annex 2. Informed consent ................................. 148
  Annex 3. Patient chart ....................................... 153
  Anexa 4. FINDRISC questionnaire....................... 155
  Anexa 5. SF-36 questionnaire ............................. 156

Key words: subclinical atherosclerosis; overweight; obesity; asymptomatic; cardiovascular diseases; cardio-metabolic risk; SCORE; FINDRISC; SF-36.
I.1. MOTIVATION OF THE PhD STUDY

Atherosclerosis represents the main cause that leads to the cardiovascular disease (CVD) development. Atherosclerosis and its thrombotic complications are associated with most deaths but still many persons are not aware of the disease because they are asymptomatic. In 30 – 50% of cases, the first manifestation of atherosclerosis is an acute coronary event, often fatal (1).

Although easily determined, potentially modifiable risk factors are present in over 90% of acute cardiovascular events (2). Atherosclerotic disease starts early but its progression is unpredictable and varies significantly. For the same exposure to risk factors, the atherosclerosis level and vulnerability are not similar among individuals. Also, the prevalence of risk factors in adults is high, but not all individuals with similar risk profile develop CVD over time. Thus, early and direct detection of atherosclerosis, before symptoms appear, confers major opportunity for CVD prevention.

Primary prevention guidelines recommend initial assessment and risk stratification based on traditional risk factors (SCORE in Europe or Framingham in North America) (3, 4). They identify low or high CVD risk persons, but most individuals will be classified as having intermediate risk. However, most acute cardiovascular events occur in these individuals, the predictive power of risk charts being diminished in this category (5 – 7). The limitations of current guidelines are admitted and non-invasive screening tests are recommended for early detection of atherosclerotic markers. However, the
presence of subclinical atherosclerosis will change the cardiovascular risk classification (8).

Overweight and obesity are significantly related to risk of cardiovascular events (9). Yet standardized and easily calculated, this risk factor is not included in cardiovascular risk charts although it is admitted that its presence confers an additional risk (5). Often a precursor of CVD, the overweight decrease represents a very important therapeutic and prevention target in current clinical practice. Moreover, overweight leads to disruption of energy balance as a result of wrong hypothalamic signaling leading to weight gain (10).

Many studies have tried to evaluate the prognostic value of subclinical atherosclerosis markers in asymptomatic population. The carotid ultrasound parameters, coronary calcium score (CCS) or ankle-brachial index (ABI) are the mostly used and validated. Echocardiographic evaluation in primary prevention is controversial. Markers of arterial stiffness (especially pulse wave velocity, PWV) were recently introduced into practice, with promising results. Although there is no ideal standard, various parameters are assessed for a more precise cardiovascular risk prediction, especially in at-risk populations such as overweight patients.

The new non-invasive imaging techniques to detect subclinical atherosclerosis can become a reliable and reproducible method for current practice use in asymptomatic but overweight patients. This attitude will determine risk stratification and reclassification of subjects (11). Few studies have examined simultaneously and comparative different markers of subclinical atherosclerosis, especially in primary
cardiovascular prevention. In this context, our research data can provide useful information on cardiovascular risk assessment by evaluating subclinical atherosclerosis through multiple non-invasive methods, easily applied in clinical practice and with special focus on a general population subset – overweight and obese persons.

I.2. THE OBJECTIVES OF THE STUDY

The purpose of the study was to characterize the subclinical atherosclerotic profile and the correlations with conventional cardio-metabolic risk factors of an asymptomatic urban population, consisting mainly of overweight adults.

The objectives of the study were the following:

- Determination of subclinical atherosclerosis by multiple non-invasive methods applied simultaneously and comparative.
- Comparison of subclinical atherosclerotic profile according to various anthropometric parameters.
- Comparison of subclinical atherosclerotic markers according to cardio-metabolic risk.
- Determination of traditional risk factor influence on subclinical atherosclerotic profile.
- Evaluation of cardiovascular risk according to subclinical atherosclerotic markers and their comparison with cardiovascular risk chart SCORE.
- Association between general health and subclinical atherosclerotic profile in the study population.
• Evaluation of subclinical atherosclerotic markers in relation to standard biochemical parameters.

II. MATERIAL AND METHOD

The current study was observational, cross-sectional and conducted over a 20-month period. The subjects were selected, by randomization, from the patients’ lists of 14 general practitioners from Iasi city. Of 700 subjects initially screened, 120 persons were included in the final study.

The inclusion criteria were as follows:

• Age between 35 – 75 years.
• Residence in urban area.
• NOT being diagnosed or not having followed treatment in the last 12 months for any metabolic, cardiovascular, respiratory, neurological or kidney disease – asymptomatic character.
• Women not being pregnant or in a state of confinement after birth.
• Subjects being psychologically and intellectually capable.
• Subjects having agreed to participate in this study.

The research has been approved by the Research Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" – Iași, and all the subjects have signed the "Informed consent" in duplicate.

All patients included in the study were completed a "Patient chart" that included:

• Personal data.
• The main cardio-metabolic risk factors: family history of CVD, personal physiological history in women, smoking, alcohol consumption.
• The evaluation of overweight by multiple parameters: weight, height, body mass index (BMI), waist circumference (WC), hip circumference, WC/hip ratio, WC/height ratio, skin fold.
• Systolic and diastolic blood pressure (SBP, DBP).
• Standard biochemical profile.
• The results of SCORE risk (for assessing CVD risk) and FINDRISC (for assessing the risk of diabetes).
• The SF-36 questionnaire results for assessing quality of life.
• The results of investigations regarding subclinical atherosclerotic parameters. Subclinical atherosclerosis was assessed by different investigations, following specific parameters:
  • Carotid ultrasound → carotid intima-media thickness (cIMT) and carotid plaques.
  • Ankle-brachial index (ABI).
  • Arterial stiffness evaluation → PWV, central systolic blood pressure (SBPao), brachial and aortic augmentation indexes (AIXbr, AIXao).
  • Transthoracic echocardiography → left ventricular mass index (LVMI) and aortic atheromatosis.
• The database was performed in Microsoft Excel 2010, and the statistical analysis was performed using
SPSS 20.0 software, considering \( p \leq 0.05 \) as having statistical significance.

III. RESULTS

III.1. General results

The mean age was 52.01 ± 10.73 years, with the median 52. Divided by decades, the distribution was uniform. Analyzed by gender, 40 subjects (33.3%) were male. In general, the age distribution of sex differences kept the initial proportion.

Family history of CVD was present in 36 subjects (30%). Out of 80 women evaluated, 41 (51.2%) were at menopause, average age of menopause being 46.56 ± 5.96 years. Regarding smoking, data was variable: 21.66% were smokers, 18.33% former smokers, and 60% non-smoking. Chronic alcohol consumption was met in 15 people (12.5%).

Most asymptomatic subjects were overweight. 81 subjects (74.2%) had a BMI over 25 kg/m² (average BMI 28.5 ± 5.34 kg/m²). Most subjects were classified as having overweight or obesity grade 1 (65%) and only 3 subjects had morbid obesity. Average values of WC exceeded the upper normal limit, both in men (103.62 ± 10.29 cm) and females (97.2 ± 13.62 cm). 72.5% of all subjects had obesity according to WC definition.

Mean SBP and DBP were mostly normal. However, although the population was apparently healthy, 28.33% of adults had diagnostic values for arterial hypertension.
Regarding the inflammatory markers, most parameters were within normal limits. In terms of lipid markers, the population was dyslipidemic. Average total cholesterol was 209.77 ± 45.56 mg/dl and 56.6% of subjects had values above 200 mg/dl. As well, LDLc (129.96 ± 40.71 mg/dl) and non-HDLc (52.49 ± 14.47 mg/dl) were at the upper limit of normal. Better average values were recorded for HDLc (52.49 ± 14.47 mg/dl) and triglycerides (137.06 ± 81.42 mg/dl) where only one third of subjects had pathological values. Mean fasting glucose value was 97.21 ± 12.75 mg/dl, only 14.16% of subjects had blood glucose above 110 mg/dl. Also, uric acid, hepatic and renal markers were mostly normal.

Most parameters of subclinical atherosclerosis were within normal limits. Overall, cIMT was close to the upper limit (0.86 ± 0.13 mm) and 36.7% had cIMT ≥ 0.9 mm. 40% of subjects had carotid plaques. ABI was pathological only in two subjects. 20.9% of participants had increased levels of PWV ≥ 10 m/s, the average being normal (8.28 ± 1.79 m/s) and the average SBPao was 128.14 ± 21.05 mmHg. Augmentation indices had mostly normal values. 70% of participants had aortic atheromatosis and 45.76% had pathological LVMI values. Average LVMI in women exceeded the pathological limit (95.93 ± 21.18 g/m²), while for men the average was close to the upper normal limit (112.89 ± 23.36 g/m²).
III.2. Overweight and cardio-metabolic risk. Correlations with subclinical atherosclerosis

Overweight or obese subjects (BMI values ≥ 25 kg/m²) were characterized by older age (53.12 ± 10.07 vs. 48.81 ± 12.04 years, p = 0.053). Current smokers and former smokers had higher values of all anthropometric parameters, but particularly weight (p = 0.015) and WC/hip ratio (p = 0.018).

Chronic alcohol consumers presented overweight defined especially by increased weight, WC and WC/hip ratio, while BMI and skin fold had equal values between the two groups. Most parameters that define obesity were higher in subjects with family history of CVD, particularly BMI (p = 0.015) and skin fold (p = 0.014). Menopausal women had overweight defined by significant increase of all anthropometric parameters.

Overweight patients had a significantly more pronounced inflammatory profile compared to normal weight subjects. From all lipid markers, low HDLc values were associated with the increase of all markers of obesity. Triglyceride value was positively correlated with most parameters, while non-HDLc associated only with skin fold. On the other hand, total cholesterol and LDLc did not correlate with any obesity marker.

Fasting glucose values were significantly higher in the group of patients with BMI ≥ 25 kg/m² (98.91 ± 13.07 vs. 92.31 ± 10.53 mg/dl, p = 0.013). Uric acid was significantly correlated with all parameters of obesity. Patients with overweight, defined by BMI as well as by WC, showed significantly decreased GFR.
cIMT correlated significantly with weight (r = 0.22, p = 0.012), WC (r = 0.26, p = 0.004) and WC/height ratio (r = 0.18, p = 0.043), respectively WC/hip ratio (r = 0.43, p < 0.001). The presence of carotid plaques correlated only with higher values of WC/hip ratio (p = 0.021). Low value of ABI was associated only with increased skin fold values.

![Fig. 1. PWV value according to BMI grade:](image)

- 7.48 ± 1.49 m/s in class 1 – normal weight;
- 8.54 ± 1.61 m/s in class 2 – overweight;
- 8.59 ± 1.79 m/s in class 3 – obesity grade 1;
- 8.30 ± 1.82 in class 4 – obesity grade 2;
- 9.90 ± 2.40 in class 5 – obesity grade 3.

The most important parameter of arterial stiffness, PWV, presented positive associations with almost all markers of obesity, the best correlations being with WC/height ratio, BMI, WC and skin fold. As well, in overweight patients PWV was significantly increased compared to normal weight subjects, both for BMI (8.58 ± 1.70 vs. 7.48 ± 1.79 m/s, p = 0.004) and WC (8.59 ±
1.82 vs 7.51 ± 1.47 m/s, p = 0.004). Moreover, PWV had significantly increased along with increasing obesity, according to BMI classes (p = 0.049) (fig. 1).

LVMI correlated with most anthropometric markers: weight (r = 0.309, p = 0.001), BMI (r = 0.27, p = 0.002), WC (r = 0.33, p < 0.001) WC/height ratio ( r = 0.28, p = 0.002), respectively WC/hip ratio (r = 0.38, p < 0.001). The presence of aortic atheromatosis showed significant associations with all markers of obesity.

Discussions

In Romania, the scientific data obtained from observational cardiovascular and metabolic studies are few (12 – 14). The overweight data is alarming in the population and the data obtained in our study are close to the national average where 66.6% of adults present overweight: 31.9% overweight and 34.7% obese (14). The prevalence of central obesity (defined by WC) was similar to that obtained recently at national level, 73.9% (14) and similar to that reported worldwide (56% in males, 71% in females respectively) (15). The results could be different also because overweight rates were reported by estimates of anthropometric data from population health surveys, whereas in our study these data were measured during clinical examination.

Consistent with our results on a group of subjects without CVD, the WC/hip ratio showed the strongest independent relationship with cIMT, over traditional cardiovascular risk factors and exceeding the predictive value of WC and BMI (16). Visceral obesity is associated with multiple pathways involved in the pathogenesis of atherosclerosis: increased atherogenic
profile, insulin resistance, homeostatic and hemodynamic changes or excess of glucocorticoids. These might explain to an extent the stronger association of subclinical atherosclerosis with abdominal obesity indices compared to BMI (16).

Regarding the relationship PWV – obesity, the results from the literature differ. Canepa M et al. showed on 711 subjects that PWV is independently associated with visceral fat but not with other markers for obesity, including WC (17). Instead, Desamericq G et al. found no significant differences between classes of obesity and PWV, regardless of associated cardiovascular risk factors (18). Another recent study obtained even lower values of PWV and SBPao in overweight or obese patients compared to normal weight (19). Thus, the data obtained in the current study provides new information in this area through positive correlations between PWV and various anthropometric markers.

III.3. Cardio-metabolic risk. Correlations between diabetes mellitus risk and subclinical atherosclerosis

Mean FINDRISC score in the study population was 10.36 ± 4.54, ranging between 0 and 22, with the median 10.5. Most patients presented low-moderated risk of DM:

- 100 subjects (83.33%) – score < 15 points (low-moderate risk)
- 20 subjects (16.67%) – score ≥ 15 points (high risk)

FINDRISC score was positively correlated with aging (r = 0.26, p = 0.004) and gender differences were
not registered. Neither on the basis of the presence or absence of family history of CVD, there were no differences in metabolic risk score. Although FINDRISC score was increased in smokers, the difference was not statistically significant. However, the presence of menopause was associated with high score (12.12 ± 3.77 vs. 8.21 ± 4.13, p < 0.0001).

FINDRISC correlated significantly with all anthropometric parameters (p < 0.001), the best values being for BMI (r = 0.522) and WC (r = 0.526). DM score progressively increased by passing from normal BMI to obesity grades (p < 0.001) (fig. 2). By applying logistic regression, only the size of the skin fold remained independent predictor for FINDRISC score (95% CI 0.040 to 0.326; p = 0.013).

Fig. 2. FINDRISC value according to BMI grade:
- 5.84 ± 3.56 in class 1 – normal weight;
- 10.83 ± 3.42 in class 2 – overweight;
- 12.58 ± 3.63 in class 3 – obesity grade 1;
- 14.38 ± 4.59 in class 4 – obesity grade 2;
- 13.00 ± 2.64 in class 5 – obesity grade 3.
Among the inflammatory markers, none showed significant associations with FINDRISC. Instead, except for HDLc, DM score correlated positively with all lipid parameters. Also, FINDRISC was strongly associated with fasting glucose levels (p < 0.001) and uric acid (p = 0.001) and a negative correlation was highlighted for GFR (r = -0.32, p < 0.001).

cIMT correlated with FINDRISC score, but not statistically significant (r = 0.169, p = 0.064). However, the presence of carotid plaques was associated with increased FINDRISC: 9.16 ± 4.49 – no plaques, 10.26 ± 4.03 – unilateral plaque, 12.24 ± 4.66 - bilateral plaques, p = 0.022. ABI showed no association with FINDRISC.

Increased risk of DM correlated with increased LVMI (r = 0.22, p = 0.017). Favorable statistical association was obtained for aortic atheromatosis as well: FINDRISC score 11.33 ± 4.21 vs. 8.00 ± 4.51, p < 0.001.

In terms of aortic stiffness markers, FINDRISC correlated statistically only with PWV (r = 0.25, p = 0.007). The other parameters had positive, but not significant correlations.

By applying and comparing subclinical atherosclerotic parameters by ROC curves, none of the markers remained statistically significant for the association with FINDRISC score. The best predictive values were for aortic atheromatosis (AUC 0.642; 95% CI 0.503 to 0.781; p = 0.093) and PWV (AUC 0.616; 95% CI 0.480 to 0.752; p = 0.171).
Discussions

If the link between type 2 DM risk and some cardio-metabolic risk factors has been studied, the relationship between DM risk and subclinical atherosclerosis has been little investigated and were not taken into account multiple parameters evaluated simultaneously, especially in primary prevention.

Adiposity correlated with PWV, possibly due to high insulin resistance and excess of free fatty acids that contributes to vascular stiffness by increasing the adrenergic reactivity, the vascular tone and the blood pressure. In the study by Hegazi RA et al., cIMT correlated with hyperglycemia, but not with obesity and insulin resistance, while the carotid plaque index (a marker of advanced atherosclerosis) was associated with the presence of inflammatory markers (20).

The results of a prospective study on 1,500 participants showed that diabetic and prediabetic subjects had higher cIMT compared to non-diabetics, suggesting that atherosclerotic damage is significant even before the clinical onset of DM (21).

Using also Arteriograph® device to determine arterial stiffness, Lenkey Z et al. showed increased PWV in patients with DM compared to non-diabetics (9.7 ± 1.7 vs 9.3 ± 1.5 m / s, p < 0.05), but AIXao was not different (22). In our study, only PWV was associated with FINDRISC, the other markers of subclinical atherosclerosis having no correlations with this score.
III.4. Quality of life, cardio-metabolic risk factors and subclinical atherosclerosis

Table I presents the means and standard deviations of the 8 scales obtained by applying the SF-36 questionnaire. By summing the scales and applying the normative data, the two main components were obtained: PCS (46.26 ± 7.75) and MCS (46.90 ± 9.78).

No parameter of the SF-36 questionnaire did correlate with age. Also, family history of CVD, smoking or chronic alcohol consumption were not associated with any category from the questionnaire. Women had lower levels of health on all scales, but statistically significant only for PF (68.17 ± 22.13 vs. 78.78 ± 19.45, p = 0.015) and MH (64.86 ± 18.98 vs 71.78 ± 16.41, p = 0.049).

Table I. SF-36 scores obtained in the study population

<table>
<thead>
<tr>
<th></th>
<th>PF</th>
<th>RP</th>
<th>BP</th>
<th>GH</th>
<th>VT</th>
<th>SF</th>
<th>RE</th>
<th>MH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Med</td>
<td>71.7</td>
<td>68.9</td>
<td>59.0</td>
<td>59.5</td>
<td>57.6</td>
<td>75.5</td>
<td>68.7</td>
<td>67.1</td>
</tr>
<tr>
<td>SD</td>
<td>21.7</td>
<td>33.7</td>
<td>24.4</td>
<td>18.5</td>
<td>17.6</td>
<td>20.2</td>
<td>36.0</td>
<td>18.3</td>
</tr>
<tr>
<td>Cαc</td>
<td>0.85</td>
<td>0.72</td>
<td>0.87</td>
<td>0.75</td>
<td>0.76</td>
<td>0.65</td>
<td>0.69</td>
<td>0.77</td>
</tr>
</tbody>
</table>

BP = bodily pain; Cαc – Cronbach’s alpha coefficient; GH = general health; MCS = mental component summary; MH = mental health; PCS = physical component summary; PF = physical functioning; RE = role-emotional; RP = role physical; SD = standard deviation; SF = social functioning; SF-36 = the Short Form 36 Health Survey; VT = vitality

Menopausal women had lower values of all variables of the SF-36 questionnaire compared to premenopausal women, the biggest differences being for:

- RP: 61.18 ± 33.74 vs. 76.39 ± 29.84, p = 0.044;
- GH: 54.42 ± 16.80 vs. 64.08 ± 18.44, p = 0.021;
- RE: 58.77 ± 39.84 vs. 76.85 ± 30.67, p = 0.033.

The lower values of the SF-36 parameters were negatively correlated with elevated BMI values, especially with RP (r = -0.22, p = 0.021), GH (r = -0.20, p = 0.026) and PCS (r = -0.26, p = 0.005). Furthermore, by dividing BMI by classes, PCS presented downward trend along with increased BMI, from 50.59 ± 7.39 in normal weight patients to 41.51 ± 9.35 in patients with morbid obesity (p = 0.023). The other anthropometric markers showed no significant associations with life quality determinants.

Table II. Differences of the health quality parameters in the presence of subclinical atherosclerosis

<table>
<thead>
<tr>
<th>SF-36 Scale</th>
<th>PWV &lt; 10 m/s (n=89)</th>
<th>PWV ≥ 10 m/s (n=22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>73.10 ± 21.98</td>
<td>65.00 ± 19.93</td>
<td>0.12</td>
</tr>
<tr>
<td>RP</td>
<td>72.55 ± 32.95</td>
<td>51.32 ± 32.78</td>
<td><strong>0.012</strong></td>
</tr>
<tr>
<td>BP</td>
<td>59.75 ± 24.96</td>
<td>55.37 ± 21.70</td>
<td>0.470</td>
</tr>
<tr>
<td>GH</td>
<td>61.11 ± 18.68</td>
<td>52.11 ± 16.54</td>
<td><strong>0.044</strong></td>
</tr>
<tr>
<td>VT</td>
<td>58.04 ± 17.33</td>
<td>55.79 ± 19.38</td>
<td>0.610</td>
</tr>
<tr>
<td>SF</td>
<td>77.31 ± 18.89</td>
<td>67.11 ± 24.72</td>
<td><strong>0.045</strong></td>
</tr>
<tr>
<td>RE</td>
<td>71.74 ± 35.26</td>
<td>54.39 ± 37.20</td>
<td><strong>0.048</strong></td>
</tr>
<tr>
<td>MH</td>
<td>68.09 ± 17.82</td>
<td>62.74 ± 20.83</td>
<td>0.25</td>
</tr>
<tr>
<td>PCS</td>
<td>46.85 ± 7.85</td>
<td>43.38 ± 6.63</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>MCS</td>
<td>47.50 ± 9.14</td>
<td>43.97 ± 12.30</td>
<td>0.15</td>
</tr>
</tbody>
</table>

BP = bodily pain; GH = general health; MCS = mental component summary; MH = mental health; PCS = physical component summary; PF = physical functioning; PWV = pulse wave velocity; RE = role-emotional; RP = role physical; SF = social functioning; SF-36 = the Short Form 36 Health Survey; VT = vitality

cIMT, carotid plaques, LVMI or aortic atheromatosis were not associated with significant
changes of SF-36. Most parameters of SF-36 were positively correlated with ABI but significant only for PF (r = 0.24, p = 0.011), suggesting a better quality of life associated with higher values of ABI.

Instead, arterial stiffness parameters had better correlations with the health level. By dividing the subjects into 2 groups according to the pathological limit of PWV, quality of life was significantly reduced in the group with increased arterial stiffness (Table II).

Including these last parameters as predictive models for pathologic PWV in the ROC curve, RP (AUC 0.315; 95% CI 0.190 to 0.441; p = 0.012), GH (AUC 0.346; 95% CI 0.222 to .471; p = 0.037) and PCS (AUC 0.334; 95% CI 0.205 to 0.464; p = 0.025) remained statistically significant.

Discussions

By comparing our results to the general norms of the Romanian population obtained by Mihaila V et al. in 2001 (23), the results were mostly similar except for PF which was significantly lower in our study (71.71 vs. 76.51, p = 0.03) and MH status that acted better in our cohort of asymptomatic individuals (67.17 vs. 61.19, p = 0.001).

We have proved that general health decreases constantly as obesity (best expressed through BMI) increases. This is applicable particularly on the physical component (RP, PCS), logically explained by the physical limitations caused by overweight. These results are comparable to those in the literature. Tan ML et al. proved on nearly 5,000 patients that overweight and obesity (evaluated by BMI, WC and WC/height ratio)
were associated with PCS score only in women while men showed a negative association between MCS and obesity (24).

I have compared the health status with multiple methods for determining subclinical atherosclerosis. Out of all, only elevated PWV values correlated with a decrease in quality of life. Asymptomatic status, relatively young age and low-intermediate cardiovascular risk could explain these results. However, the increased arterial stiffness could be a better predictive factor than other markers for life quality changes.

The relationship obtained in our study regarding the association between increased arterial stiffness and decreased parameters of life quality has been highlighted by Tiemeier H et al., but on a group of older subjects. He has shown that patients with increased arterial stiffness are more prone to depressive symptoms and each additional standard deviation to PWV determined a risk increase by 17% (25). Thus, the existence of a relationship between vascular factors and depression is suggested.

III.5. Subclinical atherosclerosis vs. cardiovascular risk SCORE

Mean SCORE value was $2.95 \pm 2.71$, ranging between 1 and 13. For a practical approach, subjects were divided into two risk categories:

- Low – intermediate risk (SCORE < 5%) – 92 subjects (76.67%);
• High – very high risk (SCORE ≥ 5%) – 28 subjects (23.33%).

Age, elevated blood pressure, chronic alcohol consumption were associated with higher cardiovascular risk. Among anthropometric parameters, only WC in women and WC/hip ratio had significantly higher values in the group of patients with SCORE ≥ 5. From all lipid parameters, only hypertriglyceridemia was associated with increased cardiovascular risk. Moreover, elevated fasting glucose, uric acid and low levels of kidney function were more common in subjects at high risk.

The overall incidence of subclinical atherosclerosis assessed in three different territories (cIMT, LVMI and PWV) was alarming. Of 120 patients, 77 (64.1%) had at least one marker of atherosclerosis.

In patients at low cardiovascular risk, only 25% had pathological cIMT, while in the group of high risk patients, 75% of them had cIMT ≥ 0.9 mm (p < 0.001). In the high cardiovascular risk group, 75% of patients had carotid plaques compared to 29.3% in the SCORE < 5 group. SCORE value increased progressively with the development of carotid plaques: 1.33 ± 0.96 in the absence of plaques, 4.43 ± 2.95 in the presence of unilateral plaques, respectively 4.80 ± 3.42 in the presence of bilateral plaques, p < 0.001.

Overall LVMI average was associated with elevated SCORE values, but the statistical significance was lost for LVMI divided by sex. Aortic atheromatosis was significantly present in the group with SCORE ≥ 5% where 100% of patients had this echocardiographic change. ABI value was not different between the two groups.
In subjects with high cardiovascular risk, all parameters of arterial stiffness had significantly higher values, the best results being for PWV and SBPao.

In multivariate logistic regression, cIMT and PWV remained independently associated with SCORE risk. Persons with cIMT values ≥ 0.9 mm had a 4.1 time higher risk for being at increased cardiovascular risk. Furthermore, each increase of 1 m/s of PWV determined an additional risk of 41% for high SCORE values. The ROC predictive value of the statistical model had an AUC 0.713 (95% CI 0.589-0.838) with 92.7% specificity and 50% sensitivity.

A positive linear relationship was shown between the SCORE risk and subclinical CVD determined in 3 different territories (cIMT, PWV, LVMI) (linear p < 0.0001). In subjects without atherosclerosis, the cardiovascular risk was 1.78 ± 1.65. Persons with 1 marker of atherosclerosis had a risk 2.71 ± 2.56, those with 2 markers of atherosclerosis had a risk 4.50 ± 3.26, and in those with all 3 markers present, the risk was 5.00 ± 2.58 (fig. 3).

89% of subjects with SCORE ≥ 5 had positive markers of atherosclerosis. However, in the SCORE < 5 group, 60% had subclinical CVD (39% in 1 territory, 18% in 2 territories, 3% in all 3 territories).

**Discussions**

In our study, cIMT was the parameter that correlated best with SCORE risk. According to the study conducted by Karim R et al., cIMT correlated independently with the cardiovascular risk at 10 years (p = 0.0003), and 69% of subjects classified as having low
risk had subclinical atherosclerosis on three different vascular territories (carotid, coronary or aortic) (26). Our results are similar except that we have evaluated the subclinical changes at the carotid, aortic and left ventricle level.

![Fig. 3. The trend of SCORE risk according to the number of subclinical CVD markers](image)

The presence of carotid plaques is variable in the general population. In our study, we have reported an overall incidence of 40%, with 25% prevalence in individuals with low and intermediate SCORE risk. In a cohort of individuals with low and intermediate cardiovascular risk (SCORE = 1.47; cIMT = 0.74 mm), 25.1% had carotid atherosclerosis and the prevalence increased along with higher SCORE values (27).

A study on subjects with no history of heart disease or stroke showed elevated PWV values significantly associated with increased CCS (p = 0.0003)
and decreased ABI (p = 0.02), suggesting target organ damage as potential mechanism associated with arterial stiffness (28). Our results confirm the positive correlation between PWV and SCORE risk and we can assume that in asymptomatic subjects with low-intermediate risk, elevated PWV values unmask the presence of atherosclerosis in other territories.

The increased prevalence of LVH in the present study may have different explanations: the assessment of undiagnosed hypertensive subjects but with blood pressure in normal range at the time of the visit or the existence of other factors that have not been assessed but could affect the left ventricular mass (increased levels of physical activity, the presence of sleep apnea syndrome).

The very low prevalence of pathological ABI in the present study can be explained by the low risk profile of the population. Similar results were obtained by Bjerrum IS et al. on a group of healthy adults where 1% of individuals had ABI < 0.9 (29).

III.6. Correlations biomarkers – subclinical atherosclerosis

The presence of uni or bilateral carotid plaques was associated with hypercholesterolemia (p < 0.001), hyperglycemia (p = 0.02), hyperuricemia (p < 0.001) and decreased renal function (p < 0.001).

Of lipid parameters, HDLc did not correlate with any marker of subclinical atherosclerosis, while increases in total cholesterol, non-HDLc and triglycerides had the best associations with high levels of atherosclerosis. cIMT and PWV correlated with uric acid
and GFR. Nonetheless, cIMT and PWV had the most positive correlations with biomarkers, while LVMI had weak associations overall.

Subsequently, we have analyzed the predictive ROC curves for cIMT, LVMI, PWV and aortic atheromatosis according to the most important biochemical markers.

For cIMT, triglycerides (AUC 0.684; 95% CI 0.584 to 0.784; p = 0.001), non-HDLc (AUC 0.611; 95% CI 0.506 to 0.717; p = 0.046) and uric acid (AUC 0.729; 95% CI 0.635 to 0.824; p < 0.0001) have significantly predicted a cIMT ≥ 0.9 mm.

For LVMI, only the value of triglycerides (AUC 0.627; 95% CI 0.525 to 0.730; p = 0.020) significantly predicted pathological LVMI.

For PWV, only GFR (AUC 0.354; 95% CI 0.231 to 0.477; p = 0.032) significantly predicted PWV ≥ 10 m/s.

**Discussions**

In our study, the lipid parameters correlated especially with markers of aortic and carotid subclinical atherosclerosis, cIMT and LVMI. On a group of adults without clinical CVD, hypercholesterolemia was associated with an increase of cIMT by 0.16 mm and the probability of coronary artery disease increased by 22% in subjects with hypercholesterolemia (30).

Inflammatory status assessed by fibrinogen level correlated only with PWV out of all markers of subclinical CVD. In other studies on asymptomatic subjects, fibrinogen and CRP were independent predictors for increased cIMT (31, 32). Possible
explanations for the insignificant correlations of inflammatory markers with subclinical CVD may be due to the low global cardiovascular risk plus the observational nature of the study.

Serum uric acid correlated with most determinants of subclinical atherosclerosis. On a population with similar cardio-metabolic profile, hyperuricemia was an independent risk factor for subclinical atherosclerosis, each unit increase in uric acid causing a 22% increase of CCS (33).

Decreased renal function was associated with aortic and carotid atherosclerosis and with elevated values of arterial stiffness. The results from the literature are divergent regarding the association GFR – cIMT (34 – 36). The association between GFR and PWV in asymptomatic population has been little studied. In the study by Miyatake N et al., GFR was negatively correlated with PWV (r = -0.308, p < 0.0001 for men, respectively r = -0.293, p < 0.0001 for women), values close to those obtained in our study (r = -0.29, p = 0.002 overall) (37). These results suggest a causal link between mild renal impairment and increased arterial stiffness even in asymptomatic subjects.

**IV. CONCLUSIONS**

- In the assessed asymptomatic adult population, 74% of patients were overweight (35% – overweight; 39% – obese).
- Most subjects were dyslipidemic.
- At least one marker of subclinical atherosclerosis was present in 64% of the study population. In
patients with low – intermediate cardiovascular risk, 60% had subclinical CVD compared to 89% of patients from the high – very high risk class. Thus, we recommend the screening for subclinical atherosclerosis in all asymptomatic individuals who have at least one risk factor, such as overweight.

- Only cIMT and PWV maintained the predictive value for increased SCORE risk. The detection of subclinical atherosclerosis in multiple territories resulted in a gradual increase in SCORE risk.
- There is no certain anthropometric parameter that has the best associations with classical risk factors. cIMT was associated with increased abdominal obesity, but not with BMI. Instead, WC and BMI were positively associated with arterial stiffness, LVMI and aortic atheromatosis.
- FINDRISC score was higher in patients with aortic atheromatosis, with carotid plaques, pathological LVMI and increased PWV, while cIMT did not correlate with the risk of DM.
- Among lipid parameters, the best associations with the markers of subclinical atherosclerosis were obtained for triglycerides.
- Among markers of subclinical atherosclerosis, only increased arterial stiffness correlated with multiple parameters of life quality questionnaire SF-36.
- ABI was the subclinical atherosclerotic parameter with the weakest correlations in the study population.
cIMT was the marker of subclinical atherosclerosis that had the best and most consistent associations with the majority cardio-metabolic risk factors. PWV had also good associations with the risk factors analyzed. In current practice, we recommend to perform at least one of these investigations for cardio-metabolic risk stratification in overweight persons.
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