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PHD Thesis

The role of new serum and imaging biomarkers in predicting the risk of acute coronary syndromes

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Summary

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Background/Introduction

Cardiovascular disease remain the main cause of mortality and morbidity worldwide according to the World Health Organization. Acute coronary syndromes (ACS) are the most severe consequence of coronary atherosclerosis and are frequently caused by plaque rupture, which, under various circumstances becomes unstable. The concept of **vulnerable plaque** (VP) has been introduced more than 30 years ago in an attempt to characterize atherosclerotic lesions that are prone to cause an acute event. Nevertheless, in order to identify patients at risk of developing adverse cardiovascular events, several other parameters should be used in an integrated approach that encompasses several other systemic factors, in addition to the local ones that characterize the vulnerable plaque. Therefore, the concept of "**vulnerable patient**" has been developed to include the presence of vulnerable plaques, vulnerable blood (with increased thrombogenicity) and vulnerable myocardium (arrhythmogenic). Numerous biomarkers have been proposed for characterization of VPs, illustrating an increased inflammatory status, which is associated with an increased vulnerability of patients with coronary heart disease. Integrating such biomarkers in **multi-omic systems** could increase their diagnostic and predictive capacity for adverse events. The development and validation of multi-omic systems is still under research and may have a crucial role in identifying patients at high risk of future adverse events, and also in creating new therapies for plaque stabilization and slowing the atherosclerotic process at a systemic level.

The aim of the present thesis was to evaluate the association between vulnerability markers (serum biomarkers for systemic vulnerability and imaging markers for characterizing vulnerable plaques), and the risk of major adverse cardiovascular events (MACE), during the course of different follow-up periods, in patient with stable coronary artery disease (CAD) and also in subjects with ACS, in a multi-marker approach that characterizes the vulnerable cardiovascular patient. The imaging markers analyzed in the present research include VP characteristics derived from coronary computed tomography angiography (CCTA) (anatomy, morphology, composition and vulnerability degree), and also for invasive coronary angiography and left ventricular systolic function quantified with transthoracic echocardiography. The analyzed serum biomarkers that illustrate the systemic inflammatory response, indicative for the vulnerable blood, include the highly sensitive C reactive protein (hs-CRP), interleukin-6 (Il-6), matrix metalloproteinase 9 (MMP-9) and adhesion molecules (E-selectin, I-CAM, V-CAM).

Materials and methods

This was a prospective observational study that included a total number of 370 patients (145 with stable CAD, and 225 with acute myocardial infarction), with a total follow-up period of 4 years, in 3 main substudies:

(1) **the first substudy** – cross-sectional, included 75 patients with stable CAD who underwent CCTA and plauque analysis, with at least one VP, with intermediary degree of stenosis, which aimed to evaluate the site-specific phenotype of VP according to its location in the three main branched of the coronary tree;

(2) **the second substudy** – case-control, included 70 patients with stable CAD who underwent CCTA and plaque analysis, with at least one VP with intermediary degree of stenosis, who were followed up for 3 years for the occurrence of ACS (defined as unstable angina, STEMI and NSTEMI) – comparative analysis between patients that had presented ACS (n=50) and who had not presented ACS (n=20) during follow-up (age and gender matched);

(3) **the third substudy** – cohort, included 225 patients with AMI (STEMI + NSTEMI) who underwent percutaneous revascularization according to the current ESC guidelines for myocardial revascularization, in which blood samples were drawn at one hour following admission in order to evaluate the serum levels of hs-CRP, IL-6, E-selectin, I-CAM, V-CAM, MMP-9; imaging markers included the severity of CAD assessed with invasive coronary angiography and left ventricular ejection fraction - LVEF (during day 5); primary end-point – MACE during a follow-up of 12 months.

The study procedures were conducted according to the ethical principles stipulated in the Declaration of Helsinki, all patients consented to the use of their data for clinical research, and the study protocol was approved by the Ethics Committee of the UMPhST of Targu Mures (no.347/13.12.2017). The statistical analysis was performed by using GraphPad Prism version 8.4.3 and MedCalc software and the statistical significance of the study was set at an alpha of 0.05.

Results

(1) Sub-study 1: Site-specific Phenotype of Atherosclerotic Lesions According to Their Location Within the Coronary Tree – a CCTA-based Study of Vulnerable Plaques

The RCA exhibited significantly longer VPs (p = 0.001), with the largest volume (p = 0.0007) compared to those arising from the LAD and CXA. Vulnerable plaques located in the LAD exhibited a significantly more calcified phenotype (calcified volume: LAD – 44.07 ± 63.90 mm3 vs. CXA –12.40 ± 19.65 mm3 vs. RCA – 33.69 ± 34.38 mm3, p = 0.002). Plaques from the RCA presented a more non-calcified phenotype, with the largest non-calcified (p = 0.002), lipid rich (p =0.0005), and fibrotic volumes (p = 0.003). Low-attenuation plaques were most frequent in the RCA (p = 0.0009), while the highest vulnerability degree was present in lesions located in the LAD, which presented the highest number of vulnerability markers per plaque (p = 0.01).

(2) Sub-study 2: The CCTA phenotype of precursor vulnerable plaques that trigger acute coronary syndromes – a 3 year follow-up study

VP that trigger an ACS present a significantly higher remodeling (1.11±0.22 vs. 0.89±0.19, p=0.002) and eccentricity index (0.54±0.25 vs. 0.3±0.12, p=0.002), higher calcified volume (110.4±130.0 vs. 35.45±77.1, p<0.0001) and also a higher lipid rich volume (13.14±12.78 vs. 2.68±1.03, p=0.0002) compared to VP that had presented a clinically silent course during follow-up. VP that had triggered ACS presented significantly higher rates of low attenuation plaques (58% vs. 10%, p=0.0007) and positive remodeling (52% vs. 15%, p=0.01), the frequency of napkin ring sign was not significantly different (p=0.4) and VP that remained clinically silent presented significantly higher rates of spotty calcifications (26% vs. 65%, p=0.005). Multivariable analysis revealed that PR, LAP and the presence of over 2 CCTA vulnerability markers per lesions were independent predictors of ACS. Most frequent location of VP triggering ACS was the proximal LAD, and right coronary dominance. VP triggering STEMI presented higher calcified and lipid rich content (p=0.01). VP triggering NSTEMI presented the highest number of VM per lesion (p=0.01). VP that had led to an ACS earliest were longer (p=0.01), with higher calcified content per lesion (p=0.005) and highest lipid rich volume (p=0.02).

(3) Sub-study 3: Predictive capacity of inflammatory and imaging biomarkers for predicting the risk of MACE in patients with revascularized AMI – a 12 months follow-up study

The MACE rate was 24.8% (n = 56). There were no significant differences between groups in regard to IL-6, V-CAM and E-selectin. The following inflammatory markers were significantly higher in MACE patients: hs-CRP (11.1±13.8 vs. 5.1±4.4 mg/L, p = 0.03), I-CAM (452±283 vs. 220.5±104.6, p = 0.0003) and MMP-9 (2255±1226 vs. 1099±706.1 ng/mL p = 0.0001). The most powerful predictor for MACE was MMP-9 of >1155 ng/mL (AUC-0.786, p < 0.001) even after adjustments for diabetes, LVEF, acute phase complications and other inflammatory biomarkers. For STEMI, the most powerful predictors for MACE included I-CAM > 239.7 ng/mL, V-CAM > 877.9 ng/mL and MMP-9 > 1393 ng/mL.

Conclusions

The present thesis has shown the association between vulnerability markers (serum biomarkers for systemic vulnerability and imaging markers for vulnerable plaque characterization) and the risk of MACE over the course of different follow-up periods, in patients with stable CAD and also with AMI, in a multi-marker approach illustrative for the concept of vulnerable patient. VP located in the RCA are longer, larger in volume with a higher lipid and non-calcified content, while those located in the LAD present a higher calcified volume and a higher vulnerability degree. The least vulnerable plaques were located in the circumflex artery. VP that precede an ACS present a soft phenotype with increased lipid rich content with positive remodeling and a higher number of vulnerability markers, in comparison to VP that remain silent over time, which present a higher fibrotic content, with spotty calcifications. PV that trigger ACS are more often located proximally and in the LAD, and are not influenced by the coronary dominance. VP that trigger STEMI present higher lipid rich volumes, higher degree of stenosis, and more often present the napkin ring sign. In addition, VP that had lead to an ACS earliest, presented an increased calcified and lipid rich volume, with an increased vulnerability degree illustrated by NRS and SCs, and >2VM per lesion.

Systemic vulnerability quantified by serum levels of inflammatory biomarkers predicts the risk of adverse events at one year in patients with revascularized AMI. High levels of I-CAM and MMP-9 were the most powerful predictors for recurrent events after AMI for the overall study population. For STEMI subjects, the most important predictors included increased levels of I-CAM, V-CAM and MMP-9, while none of the analyzed parameters had proven to be predictive for NSTEMI. Inflammatory biomarkers assayed during the acute phase of AMI presented a more powerful predictive capacity for MACE than the LVEF.