Serum ceramides as prognostic biomarkers of large thrombus burden in patients with STEMI: a micro-Computed Tomography study

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Abstract

ST-elevation myocardial infarction (STEMI) remains one of the leading causes of morbidity and mortality worldwide. The identification of novel metabolic and imaging biomarkers could unveil key pathophysiological mechanisms at the molecular level and promote personalized care in patients with acute coronary syndromes. We studied 38 patients with STEMI who underwent primary percutaneous coronary intervention and thrombus aspiration. We sought to correlate serum ceramide levels with micro-CT quantified aspirated thrombus volume and relevant angiographic outcomes, including modified TIMI thrombus grade and pre- or post-procedural TIMI flow. Higher ceramide C16:0 levels were significantly, but weakly correlated with larger aspirated thrombus volume (Spearman r=0.326, p=0.046), larger intracoronary thrombus burden (Nagelkerke R^2=0.236, p=0.030) and worse pre- and post-procedural TIMI flow (Nagelkerke R^2=0.210; p=0.049 and Nagelkerke R^2=0.277; p=0.039, respectively). Ceramides C24:0 and C24:1 were also significantly associated with larger

intracoronary thrombus burden (Nagelkerke R^2=0.311; p=0.008 and Nagelkerke R^2=0.423; p=0.001, respectively). In conclusion, serum ceramide levels (mainly C16:0 and C24:1) were higher among patients with larger intracoronary and aspirated thrombus burden. This suggests that quantification of serum ceramides might improve risk-stratification of patients with STEMI and facilitate a more individualized approach in everyday clinical practice.

Keywords: ST-elevation myocardial infarction, Thrombus, Micro-CT, Thrombus aspiration, ceramides

Introduction

Large thrombus burden (TB) in patients with ST-elevation myocardial infarction (STEMI) constitutes an independent risk factor for mortality and for adverse clinical and angiographic outcomes, including distal embolization, no-reflow phenomenon and stent thrombosis[1–4]. Although the routine use of manual aspiration thrombectomy (MATh) is not recommended in patients with STEMI according to the most recent European Society of Cardiology guidelines, patients with large preprocedural TB could benefit from MATh[5,6].

The integration of novel biomarkers, derived from patients' metabolomic profiling could provide complementary prognostic information, thereby improving risk-stratified patient management[7]. Ceramides constitute members of the sphingolipid family, which support the structure of the membrane of eukaryotic cells and mediate multiple cell-signaling pathways. Emerging evidence suggests that aberrant accumulation of ceramides has been linked to the development and progression of atherosclerosis[8]. Recent data also support their role as determinants of plaque components and predictors of plaque rupture in patients with STEMI[9], rendering these bioactive sphingolipids useful indicators for STEMI risk-stratification.

In parallel, advances in cardiovascular imaging facilitate the quantification of characteristics, which have been subjective to date[10–13]. Identification of novel imaging parameters enables patient-specific predictions of adverse outcomes[14]. Current research has demonstrated the potential for using micro-CT to quantitatively and qualitatively assess extracted thrombotic material characteristics in STEMI.

In this paper we used data from micro-CT scans of extracted thrombi from patients with STEMI. We correlated the derived volumetric findings with levels of serum ceramides, aiming to open the door for a novel personalized approach in patients with STEMI.

Materials and methods

The QUEST-STEMI study enrolled 113 STEMI patients to assess aspirated thrombus burden characteristics with micro-CT and to explore potential associations with adverse angiographic and electrocardiographic outcomes[15]. A subset of 38 patients was simultaneously enrolled in the CorLipid trial, which aims to evaluate the diagnostic utility of patients' metabolic signature for the determination of the severity of coronary artery disease (ClinicalTrials.gov: NCT04580173).

The present analysis examined patients who were co-enrolled in both studies. We sought to explore the correlation of serum ceramide levels with aspirated thrombus volume (as quantified via micro-CT SkyScan 1172), as well as with angiographic outcomes in STEMI patients undergoing primary PCI and MATh. For enhanced micro-CT analysis of aspirated clots (Figure 1), phosphotungstic acid was used as a contrast agent for their staining. The detailed protocol of micro-CT scanning has been previously described[16].

Figure 1.
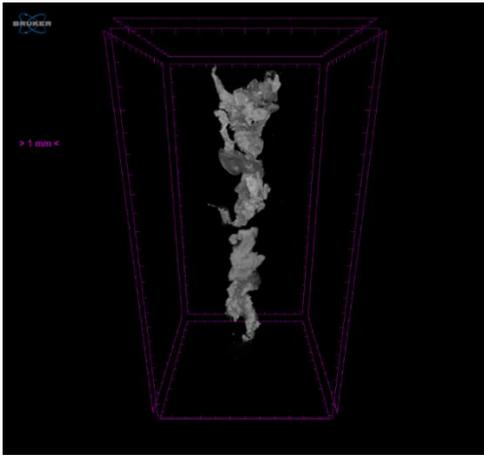
3-D volume rendering of a thrombus using the Skyscan 1172 micro-CT scanner at Hellenic Centre

for Marine Research. The clot was stained using 0.3% phosphotungstic acid as a contrast agent.

Projection images were reconstructed into sections (cross-section images) via NRecon (Bruker,

Kontich, Belgium) software.

Venous blood sample was collected from all participants prior to coronary angiography.

Ceramide species C16:0, C18:0, C24:0 and C24:1 were quantified via Ultra High Pressure Liquid

Chromatography- tandem Mass Spectrometry (UHPLC-MS/MS). Outcomes assessed were the

association of ceramides levels with: 1) aspirated thrombus volume (as quantified by micro-CT)

divided by Reference Vessel Diameter (volume/RVD), 2) the angiographic modified TIMI

thrombus grade classification[17] and 3) pre-procedural and post-procedural TIMI flow.

The association of serum ceramide levels with outcomes was assessed with use of

Spearman's correlation and logistic regression. Data analysis was executed via SPSS version 26.0

(SPSS software, Chicago, IL, USA) software and p values <0.05 were considered statistically

significant.

Results

Our results (Table 1, Figure 2) indicate a significant, but weak positive Spearman's correlation between ceramide C16:0 levels with volume/RVD values (p=0.046, r=0.326), and a non-significant trend towards the association of C18:0 and C24:1 levels with volume/RVD values (p=0.069, r=0.299 and p=0.059, r=0.309, respectively). Serum C16:0 (p=0.030, Nagelkerke R^2=0.236), C24:0 (p=0.008, Nagelkerke R^2=0.311) and C24:1 levels (p=0.001, Nagelkerke R^2=0.423) were also higher among patients with higher modified TIMI thrombus grade. Moreover, elevated C16:0 levels were significantly associated with worse preprocedural TIMI flow (p=0.049, Nagelkerke R^2=0.210). Finally, elevation in ceramide C16:0 and C18:0 levels was significantly linked with worse postprocedural TIMI flow (p values= 0.039 and 0.017, respectively).

Table 1: Association of serum ceramides levels with study outcomes

	Volume/RVD	TIMI thrombus classification	Preprocedural TIMI flow	Postprocedural TIMI flow
C16:0	**r = 0.326** **p= 0.046**	**Nagelkerke R^2= 0.236** **p= 0.030**	**Nagelkerke R^2= 0.210** **p= 0.049**	**Nagelkerke R^2= 0.277** **p= 0.039**
C18:0	r = 0.299 p= 0.069	Nagelkerke R^2= 0.119 p= 0.188	Nagelkerke R^2= 0.129 p= 0.208	**Nagelkerke R^2= 0.329** **p= 0.017**
C24:0	r = 0.214 p= 0.197	**Nagelkerke R^2= 0.311** **p= 0.008**	Nagelkerke R^2= 0.055 p= 0.601	Nagelkerke R^2= 0.218 p= 0.092
C24:1	r = 0.309 p= 0.059	**Nagelkerke R^2= 0.423** **p= 0.001**	Nagelkerke R^2= 0.177 p= 0.095	Nagelkerke R^2= 0.075 p= 0.556

Statistically significant findings (p-values <0.05) are marked in bold.

Figure 2
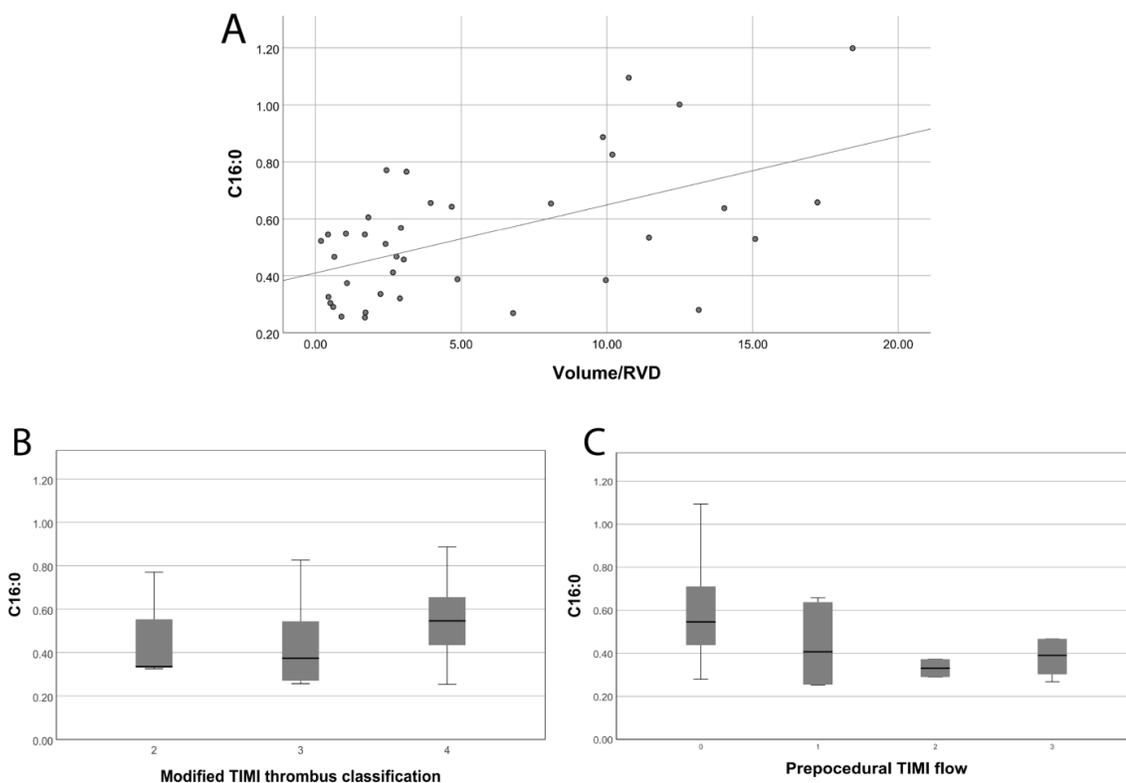
(A): Serum ceramide C16:0 levels were positively correlated with larger aspirated thrombus volume. (B): The median C16:0 level of patients with TIMI thrombus Grade 4 was higher compared to the corresponding levels of patients with lower TIMI thrombus Grades (2 and 3).

(C): The median C16:0 levels were higher in patients with worse pre-procedural TIMI flow.

Discussion

Our findings suggest that serum ceramide levels (mainly C16:0 and C24:1) are higher among patients with larger intracoronary and aspirated TB, although some correlations observed were weak. Elevated C16:0 levels were also found in patients with worse pre- and post-procedural TIMI flow. Hence, ceramides and particularly C16:0 could be potential biomarkers of high thrombotic state in STEMI patients. Consequently, they could be used as

predictive factors for the development of risk-stratification models in STEMI, with potential for evolving into a novel tool for personalized medicine.

The need for patient-level risk stratification in patients presenting with STEMI is reflected in the results of large randomized clinical trials and meta-analyses, concluding that MATh may be considered only in certain patients[18–20]. However the recent guidelines have not provided specific evidence about the profile of patients, in whom MATh should be undertaken and performing MATh is left at the discretion of the interventional cardiologist[21]. Presently, emerging evidence shows that patients with large pre-procedural TB could benefit from MATh and, therefore, ceramides could be employed as a part of a more sophisticated risk stratification algorithm, which can accurately identify patients with STEMI with potential benefit from MATh[6,22,23].

Our results are consistent with previous studies which demonstrated significant association between elevated ceramide levels and increased coronary atherosclerotic burden in STEMI patients[9,24]. Besides the proatherogenic role that ceramides may exert, studies have also documented that distinct ceramides are associated with specific plaque characteristics (plaque rupture, higher necrotic core fraction or higher lipid core burden) or increased cardiovascular and cerebrovascular risk[25–27]. However, our study was not designed to assess hard clinical endpoints and our findings should also be interpreted taking into consideration the single-center nature of the study and the restricted sample of participants.

In conclusion, quantification of serum ceramides might improve risk-stratification of patients with STEMI and guide future decision-making in a more individualized approach. Further research is warranted to explore the association of TB with ceramides levels and

elucidate whether these sphingolipid products could be employed as potential diagnostic or therapeutic targets.

Author Contributions

HG, GT and GS2 developed the concept and the methodology of the study. EK and AP wrote the manuscript. GS1 and GS2 were responsible for coronary angiography execution and angiographic outcomes assessment. EP and DM contributed to the research data management and statistical considerations. DM, NS and AB were responsible for patient recruitment and blood sample collection, whereas OD, OB and HG were responsible for the biochemical analysis

of the study. Micro-CT analysis of the aspirated thrombi was conducted by KK and EC. The supervisor of the whole study was GS2. All authors have read and approved the final manuscript (GS1 refers to Georgios Sofidis and GS2 refers to Georgios Sianos).

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Scientific Committee of AHEPA University Hospital (reference number 12/13-06-2019) and by the Directory Board of AHEPA University Hospital (reference number 17/29-08-2019).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Data are available from Georgios Sianos (e-mail: gsianos@auth.gr) upon reasonable request and with permission of AHEPA University Hospital.

Conflicts of Interest

The authors declare no conflict of interest.

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