Leukotriene biosynthesis in coronary artery disease

Results of the Leukotrienes and Thromboxane In Myocardial Infarction (LTIMI) study

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INTRODUCTION
Leukotrienes (LTs) may be involved in atherosclerosis and may contribute to cardiovascular outcomes in CAD.

OBJECTIVES We aimed to compare the baseline LT production in patients with stable CAD (sCAD) and myocardial infarction (MI), and to assess whether an increased LT production is associated with major adverse cardiovascular events (MACEs) at 1 year after MI.

PATIENTS AND METHODS LTIMI (Leukotrienes and Thromboxane In Myocardial Infarction) was a single-center, prospective, observational study of patients with stable sCAD and MI. Urinary leukotriene E4 (LTE4) levels were measured on admission, at 1 month, and at 1 year, using high-performance liquid chromatography tandem mass spectrometry.

RESULTS Of the 404 patients screened, 289 were enrolled (110 with sCAD and 179 with MI; mean [SD] age, 63.9 [10.9] years). Patients with MI had higher median (interquartile range [IQR]) levels of log-transformed LTE4 (logLTE4) than those with sCAD (4.74 pg/mg creatinine [4.39–4.86] vs 4.51 pg/mg creatinine [3.99–4.86], respectively; P<0.001). Median (IQR) logLTE4 levels in patients with MI significantly decreased at 1 month to 4.37 pg/mg creatinine (3.81–4.95), and at 1 year to 4.16 pg/mg creatinine (3.55–4.85). The baseline urinary logLTE4 levels were similar in patients with MACEs and those without MACEs (median [IQR], 4.78 pg/mg creatinine [4.01–5.56]) and 4.68 pg/mg creatinine [3.97–5.28], respectively; P>0.05). Multiple regression showed no relation between LTE4 levels and the incidence of MACEs.

CONCLUSIONS LT production assessed by urinary LTE4 excretion is higher in patients with MI than in those with sCAD; however, LTE4 levels at baseline do not differ between patients with and without MACEs at 1 year after MI.

INTRODUCTION Leukotrienes (LTs) are potent lipid mediators, derived from the 5-lipoxygenase (5-LO) arachidonic acid pathway, originally known for their role in the pathophysiology of inflammation and asthma. The primary source of LTs are myeloid cells, but considerable amounts are produced in inflammatory tissues by endothelial cells and platelets by transcellular synthesis. LT biosynthesis is a complex process initiated at the nuclear envelope by arachidonic acid release from phospholipids; it requires a set of specific enzymes and proteins, including 5-LO, 5-LO–activating protein (FLAP), LTC4 synthase, or LTA4 hydrolase. The main products of the LT cascade are LTC4, LTD4, and LTE4 due to the presence of cysteine, collectively named cysteinyl leukotrienes (CysLTs), whereas LTB4 is a potent chemotactrant of neutrophils. The final metabolite of CysLTs is LTE4, excreted in urine in an unchanged form or N-acetyl-LTE4. The measurement of the serum levels of CysLTs is not a reliable method of assessing their
biosynthesis. However, the measurement of the urinary LTE₄ concentration is a generally accepted method to estimate a systemic production of CysLTs, and hence to determine the activity of the 5-LO pathway in vivo. As a class, LTs have recently been intensively studied for their role in atherosclerosis and inflammation contributing to coronary artery disease (CAD). With the recent advent of novel anti-inflammatory strategies added on to conventional prophylaxis reducing cardiovascular risk, the LT pathway has become the strategic aim of therapeutic investigations.

The distinct role of LTs in human atherosclerosis is mostly attributed to their paracrine activities exerted through the receptors CysLTR1, CysLTR2, LTβR41, and LTβR42, which are expressed in the walls of the aorta and coronary vessels. As a consequence, LTs are believed to promote vascular inflammation via increased leukocyte chemotaxis, enhance permeability and tissue and matrix degeneration, and contribute to the risk of plaque rupture with subsequent thrombosis.

A series of preclinical studies provided some evidence that the 5-LO pathway promotes the development, progression, and destabilization of atherosclerotic plaques, leading to abdominal aortic aneurysm formation, myocardial infarction (MI), and stroke. Immunohistochemical studies proved that in atherosclerotic vascular lesions the key 5-LO pathway enzymes and receptors were expressed and correlated with the severity of the lesions and plaque instability.

The above findings have led to a hypothesis that LTs contribute to cardiovascular outcomes in CAD and MI. In a large population-based cohort study, treatment with the LT receptor antagonist montelukast was associated with a lower risk of recurrent stroke, but no further associations with cardiovascular events were found. Despite strong scientific rationale, only few studies have addressed this issue in clinical trials attempting to modify the LT production by inhibiting the 5-LO pathway. Moreover, the results of these trials are conflicting.

LTIMI (Leukotrienes and Thromboxane In Myocardial Infarction) was a prospective, observational study that aimed to assess the association between arachidonic acid derivatives (LTs and thromboxane A₂) and major adverse cardiovascular events (MACEs) in patients with CAD. In a previous publication, we reported thromboxane A₂ as a predictor of cardiovascular outcomes in MI. In the current study, we aimed to compare the baseline production of LTs in stable CAD (sCAD) and MI and assess whether the higher levels of LTs are associated with more frequent MACEs in a 1-year follow-up after MI.

PATIENTS AND METHODS We conducted a single-center, prospective, observational, pragmatic study in 2 parallel groups of patients with sCAD and MI. All patients gave informed consent to participate in the study. The study protocol complied with the Helsinki Declaration and was approved by the Ethics Committee of Jagiellonian University (Kraków, Poland).

Study population The LTIMI study was conducted between July 2011 and September 2014. Eligible patients aged between 31 and 88 years were admitted either for scheduled coronary angiography (CA) because of sCAD or were diagnosed with MI (both ST-elevation MI [STEMI] and non-ST-elevation MI [NSTEMI]) and transferred in a timely manner for revascularization. In the MI group, only patients with MI type I (atherothrombotic, spontaneous) and symptoms lasting no longer than 24 hours were eligible for the study. The type of MI was evaluated based on the universal definition of MI by 2 cardiologists that were not blinded to the clinical data of the patients, including CA findings.

The exclusion criteria for the MI group were: the lack of baseline LTE₄ measurements, late presentation of MI (>24 hours from the first symptoms), MI other than type I, cardiogenic shock, history of coronary artery bypass grafting (CABG), severe valvular heart disease, symptoms of acute infection, asthma, chronic obstructive pulmonary disease exacerbation, use of antileukotriene medications, severe chronic kidney disease (estimated glomerular filtration rate [eGFR] <30 ml/min/1.73 m²), liver cirrhosis, malignancy, patient refusal to participate in the study, and noncompliance. Patients administered antiplatelet drugs other than acetylsalicylic acid and clopidogrel prior to other interventional trial (being the most common exclusion criterion) were excluded from the study. In the sCAD group, an additional exclusion criterion was ad hoc PCI performed immediately after the diagnostic CA. The study flow chart is presented in Figure 1.

Study procedures Assessment at baseline Data on comorbidities, demographic characteristics, and history of presenting complaint were collected on admission in both groups of patients—with sCAD and MI. On admission, standard laboratory tests were performed including serial high-sensitivity troponin T measurements (hs-TnT) (Roche Diagnostics, Mannheim, Germany). Blood and urine samples were collected from each participant prior to the CA and stored immediately after centrifugation at -70°C for further analysis.

Leukotriene measurement CysLT levels were measured in urine samples. In the sCAD group, the samples were collected in the morning before CA, whereas in the MI group—on admission (the mean [SD] time from symptoms to sample collection was 9.21 [6.7] hours). Additionally, the samples were collected at 1-month and 1-year follow-ups. Urinary LTE₄ excretion was assessed with
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The results were expressed in picograms (pg) per mg urine creatinine (pg/mg creatinine).

LTE4 levels were measured at the presence of an identical deuterated standard added to the urine sample before the HPLC-MS/MS measurement. The results were expressed in picograms (pg) per mg urine creatinine (pg/mg creatinine).

 Abbreviations: CA, coronary angiography; CABG, coronary artery bypass grafting; CV, cardiovascular; LTE4, leukotriene E4; MACE, major adverse cardiovascular event; MI, myocardial infarction; NSTEMI, non–ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; sCAD, stable coronary artery disease; STEMI, ST-segment elevation myocardial infarction
**Transcatheter echocardiography** Standard transcatheter echocardiography (TTE) was performed on admission and at 1 year in all patients with sCAD and MI. Two experienced echocardiographers—cardiologists, blinded to CysLT measurement results, assessed the images independently, according to current guidelines.\(^{25}\) Left ventricular ejection fraction (LVEF) was calculated using the Simpson’s method.

**Coronary angiography and percutaneous coronary intervention** CA was performed in both study groups, using a standard technique, under local anesthesia by puncture of the femoral or radial artery, with typical diagnostic catheters. The arterial access was selected at the discretion of the operator. All patients were treated with primary PCI in the setting of STEMI, and immediate ad hoc PCI in the setting of high-risk NSTEMI. Most patients underwent an immediate invasive strategy (0–2 hours) or early invasive strategy (within 24 hours of diagnosis). No delayed invasive strategy (within 72 hours of diagnosis) was used in the setting of NSTEMI.

**Follow-up** Patients were followed for 1 year after enrollment to the study. All patients had 2 visits scheduled in an outpatient clinic: 1 month and 1 year after hospitalization. At both follow-up visits, the research team collected data on the occurrence of MACEs and verified the information based on hospital discharge notes and interviews with family members. Urine samples were collected at both visits for LTE\(_4\) analyses.

**Outcome measures** There were 2 primary outcomes of the study: 1) to compare the LTE\(_4\) production between patients with sCAD and MI; and 2) to evaluate the impact of the urinary LTE\(_4\) concentration on the occurrence of a composite MACE that occurred during 1-year follow-up after hospitalization for MI or sCAD. The composite MACE was defined as the occurrence of at least 1 of 8 MACEs, prespecified before commencement of the study, namely, recurrent MI, stroke/transient ischemic attack, cardiogenic shock, heart failure event, nonfatal cardiac arrest, need for rescue PCI or CABG, and cardiovascular death. If 2 MACEs occurred in one patient (eg, recurrent MI and death), they were counted as one outcome. The exact definitions of MACEs are provided in a previous LTIMI study publication reporting thromboxane \(A_2\) biosynthesis in MI.\(^{22}\)

**Statistical analysis** Categorical variables were presented as counts (percentages), whereas continuous variables were reported as mean (SD) or median with interquartile range (IQR), depending on their distribution. Urinary LTE\(_4\) levels were log-transformed (natural logarithms; logLTE\(_4\)) to approximate normal distribution for further analysis. Assumption of normality was verified using the Kolmogorov–Smirnov test. The differences between the following groups: MI and sCAD; MACE vs non–MACE, were tested with the t test, Mann–Whitney test, and Fisher exact test for categorical variables. Correlations between variables were analyzed using the Pearson’s or Spearman’s rank correlation, as appropriate for the data distribution. For the analysis of urinary LTE\(_4\) levels at subsequent time points, the 1-way analysis of variance for repeated measures and the post hoc Tukey test were used. For the comparisons of LTE\(_4\) with MI group, the Kruskal–Wallis test was used with the post hoc Mann–Whitney comparison adjusted with Bonferroni correction.

The association between LTE\(_4\) levels and the incidence of the composite MACE was evaluated using the multiple logistic regression model only in the MI group, because in the sCAD group there were only 4 MACEs. Explanatory variables were chosen in a stepwise approach on the basis of the Akaike Information Criterion. Regression modeling included prespecified baseline clinical and laboratory characteristics, namely, age, sex, body mass index, smoking history (pack-years), previous MI, diabetes mellitus, hypertension, multivessel disease, time from symptoms to PCI, LVEF during hospitalization, maximum hs-TnT level, high-sensitivity C-reactive protein (hs-CRP), eGFR, and logLTE\(_4\) concentrations. All tests were 2-tailed, with \(P\) values of less than 0.05 considered significant.

All statistical analyses were performed using the R software, 3.1.2. version (R Development Core Team. [2009] R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) and STATISTICA 12 (StatSoft, Inc., Tulsa, Oklahoma, United States).

**RESULTS** Study population A total of 404 patients were screened for the inclusion and exclusion criteria of the LTIMI study, and 289 patients were enrolled. The study flow chart is presented in Figure 1. In this group, 110 patients had sCAD and were admitted for diagnostic CA and the remaining 179 patients had MI type I and were referred for PCI (\(n = 162\)) or rescue CABG (\(n = 17\)). The sex distribution was similar in both groups; however, patients with MI were older (mean [SD] age, 66.26 [11.92] years vs 60.03 [7.7] years; \(P < 0.001\)), more often had diabetes, symptoms of claudication, and dyslipidemia (mean [SD] low-density lipoprotein cholesterol levels, 3.1 [1.14] mmol/l vs 2.7 [0.98] mmol/l; \(P = 0.001\)), and higher hs-CRP levels (mean [SD], 9.62 [23.1] mg/l vs 3.43 [6.6] mg/l; \(P < 0.001\)). The basic demographic and clinical characteristics of the study groups are presented in Table 1.

**Urinary leukotriene \(E_4\) excretion** The median (IQR) LTE\(_4\) row data for sCAD and MI were as follows: 91.14 (53.95–128.87) and 114.98 (55.3–232.42). All analyses were performed on log-transformed data. The LTE\(_4\) excretion did not correlate with age, sex, or the laboratory parameters such as white blood cell count, hs-CRP, and hs-TnT. On
admission, patients with MI had higher levels of log\(\text{LTE}_4\) than patients with sCAD (4.74 pg/mg creatinine [IQR, 4–5.45] vs 4.51 pg/mg creatinine [IQR, 3.99–4.86]; \(P<0.001\); FIGURE 2). This difference was irrespective of age, sex, or the effect of comorbidities in the multivariable regression model. In the MI group, there were no differences in the \(\text{LTE}_4\) production between patients with STEMI and those with NSTEMI.

**Urinary leukotriene \(\text{LTE}_4\) excretion at 1 year**  
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and 1-year follow-ups. In the MI group, the median (IQR) logLTE₄ level decreased from 4.74 pg/mg creatinine (4–5.45) to 4.37 pg/mg creatinine (IQR, 3.88–4.95) at 1 month (P < 0.05), and to 4.16 pg/mg creatinine (3.55–4.85) at 1 year (P < 0.05 compared with the level at 1 month and P < 0.0001 compared with the baseline level). The LTE₄ level at 1 month after MI was similar to the baseline LTE₄ level in patients with sCAD (P = 0.05), and at 1 year, it was lower than the baseline level in patients with sCAD (P = 0.02). In the sCAD group, the LTE₄ level was stable during 1-year follow-up (P > 0.05). The changes in LTE₄ levels in the sCAD and MI groups are shown in FIGURE 3.

**FIGURE 3** Urinary log-transformed leukotriene E₄ (logLTE₄) during 1-year follow-up in patients with stable coronary artery disease (sCAD; n = 102) and myocardial infarction (MI; n = 165). Data are presented as mean with SEM.

DISCUSSION In this study, we found that systemic CysLT production, measured by urinary LTE₄ excretion, was increased during MI, as compared with patients with sCAD. Subsequently, after an acute event, urinary LTE₄ levels decreased during 1 month and progressively during 1 year, suggesting that LTs may be involved in the pathophysiology of MI. At the same time, we showed that CysLT levels at the index hospitalization for MI did not differ in patients who suffered MACEs during 1-year follow-up, when compared with those without MACEs.

An increased LT production during acute MI has been reported in both experimental animal models and humans. The evidence for 5-LO activation was first shown by Carry et al. in a group of 16 patients with MI treated with tissue plasminogen activator and 14 patients with unstable angina. The LTE₄ level decreased on the third day after the ischemic episode. A similar increase in the urinary LTE₄ excretion was also evidenced by De Caterina et al., who measured LTE₄ levels in small groups of 16 patients with NSTEMI, 6 patients with STEMI, and 17 patients with stable angina. They found that the mean values of urinary LTE₄ levels are increased in patients in the setting of acute ischemia. Myocardial ischemia induced by a positive exercise stress test was not accompanied by an increase in detectable levels of LTE₄ in our study, while a significant increase was observed after PCI (n = 10), as compared with diagnostic CA (n = 9). This finding is consistent with a study by Rzeszutko et al., who...
TABLE 2  Multivariable logistic regression model for the prediction of major adverse cardiovascular events during 1-year follow-up, including leukotriene E4

<table>
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Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; hs-TnT, high-sensitivity troponin T; others, see FIGURE 1 and TABLE 1

observed the overproduction of urinary LTE₄ during coronary interventions in patients with CAD. In acute coronary syndromes, including unstable angina, activation of the 5-LO pathway extended up to several months. A short-term activation of the 5-LO pathway was reported after a coronary intervention in patients with sCAD, lasting a few hours after the procedure. These values returned to the baseline as early as 24 hours after the intervention. All the above reports corroborate our findings and the hypothesis that LTs may participate in the pathomechanism of acute MI. However, further studies are needed to evaluate the potential causative role of LTs in the pathogenesis of MI.

The fact that baseline LTE₄ levels are not elevated in patients who after MI present with MACEs seems not to correlate with increased baseline LTE₄ production. Several possibilities may account for this finding. This study provides clear evidence that LTE₄ levels do not correlate with cardiovascular risk factors or specific cardiac biomarkers such as high-sensitivity troponins. There is also no correlation with systemic inflammatory markers. In the current study, we determined the levels of hs-CRF, which is the most widely used inflammatory marker in cardiovascular disease and the only reproducible indicator for which a standardized assay has been developed. The inflammatory profile of our study population was carefully assessed and unified during recruitment by excluding patients with evidence of systemic inflammation. Nevertheless, we should allow for the fact that the CRP measurement has a limited specificity for atherosclerosis-related inflammation, and its prognostic value for future cardiovascular events is particularly weak, especially in patients who receive optimal medical prophylactic treatment including antiplatelet medications and statins as secondary prevention.

CysLTs represent not only proinflammatory mediators, but also potent positive modulators of vascular permeability. The increased production of LTs during episodes of acute ischemia may not be related to the inflammatory destabilization of atheromatous plaque, but may result from intravascular LT production by activated platelets and granulocytes. This transcellular mechanism of LT biosynthesis was proposed to be involved in ischemia/reperfusion injury, in which endothelial dysfunction was mediated by the CysLTR2 receptor. Any direct vasoconstrictive effect of LTs has never been demonstrated in clinical trials on acute coronary syndromes, despite a casuistic description of myocardial ischemia in a patient with extreme overproduction of LTE₄.

Preclinical models of the LT pathway in myocardial ischemia–reperfusion injury are based primarily on genetically engineered mice. Despite experimental studies on multiple targets within the LT pathways, including 5-LO, FLAP, LTC₄ synthase, or CysLT1 and CysLTR2 receptors, the impact of LTs on the pathogenesis of CAD and MI was not evidenced by unequivocal results. In particular, studies on CysLTR receptors were heterogeneous, thus raising doubts as to whether the 5-LO pathway is involved in the pathogenesis of CAD. The main concern about murine models of the LT pathway was the fast acceleration of atherosclerosis, which develops in mice within months, whereas in humans the disease progress takes decades. It was noted that the biosynthesis of the 5-LO pathway in murine atherosclerosis may be restricted only to the early phase of the disease.

Clinical genetic studies on genes determining the LT pathways also aroused some controversies. For example, certain variants of genes encoding FLAP and LTA₄ hydrolase were associated with the risk of MI and stroke in European, Japanese, and African-American populations, but they were not associated with MI and the risk of ischemic stroke in white North Americans. Two polymorphisms of the 5-LO promoter were associated with carotid intima–media thickness in the North American population, but were not associated with an increased risk of MI in the Spanish population. All these studies suggested a population-specific influence of polymorphisms in genes encoding the biosynthesis of the LT pathway, possibly explaining the lack of contribution of LTs to cardiovascular outcomes in MI.

Finally, the results of experimental studies in recent years raised legitimate hopes for the use of antileukotrienes, well known for their safety of use in the treatment of asthma as an add-on therapy to the conventional cardiovascular...
prevention. Pharmacological studies using leukotriene-synthesis inhibitors and leukotriene receptor blockers in CAD initially demonstrated promising results. The first clinical trials with DG031 (velilapen [BAYx1005], one of the FLAP inhibitors) and DG051 (LTA4 hydrolase inhibitor) for secondary prevention of MI and stroke in patients with CAD showed beneficial effects, showing a reduction in inflammation and stabilization of atherosclerotic plaques. The results of a phase 2 trial on VIA-2291 (atreluton, inhibitor of 5-LO) in patients with acute coronary syndromes (ClinicalTrials.gov identifier, NCT00352417) indicated that the compound inhibits the synthesis of LTs immediately after an episode of acute coronary event and significantly reduces the size of atherosclerotic plaques in follow-up. However, although it was found to reduce the LT production, it did not express significant anti-inflammatory properties.

In conclusion, the LTMI trial evaluated the production of CysLTs assessed by urinary LTE4 excretion. It showed that LT levels were higher in patients with MI compared with those with sCAD. This phenomenon may be important for future research into the pathogenesis of atherosclerosis and MI and is in line with findings from previous studies. However, LTE4 production does not have a prognostic value in predicting MACIs during 1-year follow-up. This new finding may explain the equivocal results of numerous preclinical studies on animal models of the LT pathway and of pharmacological trials with novel anti- leukotriene agents to reduce cardiovascular risk.

Further clinical studies are needed to define the place of CysLT in the pathophysiology of MI. A clear differentiation of the complex 5-LO pathway from cell- and tissue-specific mechanisms contributing to atherosclerosis should be considered, using additional biomarkers to address more specifically the role of systemic inflammation and the identification of atherosclerosis-related inflammation and plaque vulnerability. This approach could help determine the importance of LTs in the pathogenesis of atherosclerosis and MI in order to provide a solid basis for developing future therapies.

**Study limitations** This trial was a single-center study with a limited number of patients. The study group was sufficient to evaluate the primary composite MACE, but not large enough to evaluate each different MACE separately (which was anticipated and predefined in the study protocol). The study population included patients with STEMI and NSTEMI, which makes the group heterogeneous; however, based on the regression models, it should not have affected the results. Finally, we did not evaluate the effect of the treatment strategy on the final outcomes.

**ACKNOWLEDGMENTS** We thank Anna Gielicz for her invaluable technical support in measuring LTE4 levels and Łukasz Deryło for statistical support. We would like to acknowledge the contribution of Professor Andrzej Szczeklik, who passed away in February 2012, to the design of this study.

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**CONTRIBUTION STATEMENT** WS, MS, and KŻ conceived the idea for the study. All authors were involved in data collection. WS and MS analyzed the data. WS and MS obtained funding for the project. All authors edited and approved the final version of the manuscript.

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