

Do circulating concentrations of L-arginine metabolites predict coronary artery disease status and likelihood of cardiovascular events?

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Acute myocardial infarction (MI) secondary to coronary heart disease remains one of the leading causes of death.¹ Measurement of biochemical parameters in peripheral blood is minimally invasive, and can be technically undemanding as compared with angiography. Ideal biomarkers would be useful in predicting the disease stage and would indicate impending plaque rupture and thus increased likelihood of infarction. Unfortunately, such biomarkers have not yet been identified. In the current edition of *Polish Archives of Internal Medicine*, Mołek et al² addressed such issues and reported that, among other clinical measures, thin-cap fibroatheroma (TCFA) and increased intima-media thickness are associated with an altered balance of L-arginine (Arg) metabolites in patients with acute MI and 6 months later. In their earlier study, the authors observed a shifted balance of Arg metabolites in patients with acute MI.³

Arg is one of the most versatile amino acids in the human body.⁴ Free Arg is a precursor of numerous important biomolecules, such as nitric oxide (NO) and guanidinoacetate (GAA), and a precursor of the energy-related creatine (FIGURE 1). Arg residues in proteins undergo several post-translational modifications, notably guanidine (N^G) methylation and citrullination.⁵ Proteolysis of N^G-methylated proteins releases monomethylarginine (LMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA). These methylated Arg metabolites inhibit the activity of constitutive and inducible NO synthase (NOS) isoforms and may also interfere with the cellular Arg transport⁶ (FIGURE 1). All NOS isoforms convert Arg to NO and L-citrulline, albeit with different activity, with inducible NOS (iNOS) being the most active, followed by neuronal NOS (nNOS), and endothelial NOS (eNOS). NO is a gaseous free radical molecule,

a neurotransmitter, and one of the most potent endogenous vasodilators and inhibitors of platelet aggregation. Under physiological conditions or in the presence of medicines Arg-consuming pathways change dynamically, but stay in balance with complex cellular interactions. Valid analytical methods are required to determine deviations of “normal” values and intervals of certain metabolites in the blood, which may then be used in diagnosis and therapy. Determination of several parameters enables a more complex assessment of the imbalance caused by a disease or by other circumstances, such as pharmacological interventions.⁷ Yet, there are many difficulties and limitations in this context.

The global arginine bioavailability ratio (GABR) is a widely used composite measure in clinical studies investigating the pathways that involve Arg. GABR is a dimensionless ratio of the concentration of Arg and total concentration of its major metabolites, that is, citrulline (Cit) and ornithine (Orn) in the circulation: $GABR = [Arg]/([Cit] + [Orn])$. The GABR value of 1, which is equivalent to 100%, can be observed for low, normal, and high concentrations of Arg, Cit, and Orn, indicating in all the cases a balanced Arg pathway, yet at different levels of concentration. Temporary (over 4 h), drastic changes in GABR values (from 1 to 0.02) were observed in patients after orthotopic liver transplantation (OLT),⁸ likely due to a release of huge amounts of arginase from the liver graft.⁹ Nevertheless, despite the almost complete removal of Arg from the patients' blood, no decrease in this time window was observed in the plasma and urine concentration of ADMA and its major metabolite dimethylamine (DMA), or of nitrate and nitrite,⁸ the major NO metabolites and measures of its synthesis. This finding indicates a limited usefulness of GABR as a measure of the balance of

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to produce NO from the NOS-substrate Arg and the NOS-inhibitor ADMA.¹⁰ The Arg/ADMA ratio is subject to great variations. To the best of our knowledge, no values or intervals have been recommended so far for GABR, C/O, O/A, or the Arg/ADMA ratios in experimental or clinical studies.

A major question that arises in this area is whether concentration changes seen in the circulation for the metabolites of Arg pathways, which are ubiquitous, are specific to a particular organ, tissue, or artery segment. It seems that this is realistic in acute rather than chronic conditions, as observed for arginase in OLT.^{8,9} But are altered Arg metabolites predictors of acute and chronic coronary heart disease and outcome? Mołek et al² suggested that the cause of altered Arg metabolites may be locally increased arginase activity in ischemic and reperfused myocardium that results in impaired NO synthesis due to low Arg concentrations. However, a causal link with TCFA appears inconclusive. The authors referred to cell culture and animal studies, where increased arginase activity was observed in smooth muscle cells, the vessel wall, and in atherosclerotic plaques. However, arginase is highly expressed in the liver, small intestine, as well as in circulating monocytes, and alveolar macrophages. MI may evoke acute effects in these organs and cells, including changes in their arginase activity.¹¹ Common comorbidities in MI patients, such as diabetes,^{12,13} and medications, such as ticagrelor¹⁴ and heparin¹⁵ may also alter Arg metabolism in different mechanisms including alteration of arginase activity. Further, contribution of nutrition, a major external source of Arg, nitrite, nitrate, and DMA, is a limiting factor. Finally, TCFA were analyzed in the culprit lesions only, while patients may have suffered from multi-vessel coronary disease or peripheral atherosclerotic disease, where the conditions of these vessels are unknown.

We noted in the papers by Mołek et al^{2,3} very low circulating concentrations of Arg, Cit, Orn, and proline, and very high circulating concentrations of agmatine (AGM) and spermidine (SPD). The reported AGM and SPD concentrations were even higher than those of their precursors. In addition, extreme values of Arg metabolites were omitted in some statistical analyses of the study,² yet the reader is not aware of the origin and reasons for these deviations. Large deviations in analyte concentrations are likely to be due to analytical shortcomings, even when using highly sophisticated techniques such as liquid-chromatography tandem mass spectrometry, and they may weaken the outcome of the study. The use of validated, precise, and accurate analytical chemistry methods for the measurement of concentrations of Arg metabolites in plasma or serum is an indispensable requirement.

Special attention needs to be paid when analyzing associations of biochemical measures, such as Arg and its metabolites, with specific functional

clinical measures, such as TCFA. The statistical methods to be used in such clinical studies need to be clearly defined. They should include acceptance and rejection criteria for relevant parameters, including the extent of association of potentially new biomarkers with established specific biomarkers. The extent of minimum values for receiver operating characteristic (ROC), odds and hazard ratios also need definition alongside the acceptance and rejection criteria. In our opinion, ROC values of about 0.8 for GABR, O/A, and the other parameters are too low for serving as predictors of ischemia, even if analyses indicate statistical significance.

ARTICLE INFORMATION

DISCLAIMER The opinions expressed by the author(s) are not necessarily those of the journal editors, Polish Society of Internal Medicine, or publisher.

CONFLICT OF INTEREST None declared.

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