ASYMMETRIC DIMETHYLARGININE (ADMA): A MEDIATOR OF ENDOTHELIAL DYSFUNCTION AND A NOVEL CARDIOVASCULAR RISK FACTOR

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Asymmetric dimethylarginine (ADMA) is involved in the pathogenesis of hypertension and atherosclerosis by inhibiting the generation of nitric oxide, an endogenous vasculoprotective molecule. Determination of ADMA helps to predict a patient’s probability of experiencing death or cardiovascular disease. A novel competitive ADMA-ELISA that has recently been developed and validated by us is a useful and fully validated tool for routine laboratory use.

Role of Endothelial NO in Vascular Disease

The endothelium plays a crucial role in the maintenance of vascular tone and structure. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO), which is formed from the amino acid precursor L-arginine by nitric oxide synthase. NO is involved in a wide variety of regulatory mechanisms of the cardiovascular system, including vascular tone (i.e. it is the major mediator of endothelium-dependent vasodilation) and vascular structure (e.g. inhibition of smooth muscle cell proliferation), and cell-cell-interactions in blood vessels (e.g. inhibition of platelet adhesion and aggregation, inhibition of monocyte adhesion). Due to these functions, NO has been summarized as an "endogenous anti-atherosclerotic molecule."

Dysfunction of the endothelial L-arginine/nitric oxide pathway is a common mechanism by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall. Among them are hypercholesterolemia, hypertension, smoking, diabetes mellitus, homocysteine, and vascular inflammation.

ADMA, a Mediator of Endothelial Dysfunction

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthesis. ADMA inhibits vascular NO production within the concentration range found in patients with vascular disease; ADMA also causes local vasoconstriction when it is infused intra-arterially. Currently available experimental and clinical evidence suggests that even small modifications of ADMA significantly change vascular NO production, vascular tone, and systemic vascular resistance (for review, see [1]). This is evidence enough to call ADMA a marker of endothelial dysfunction.

ADMA, a Marker of Vascular Disease

There are numerous studies to date showing a relationship between elevated ADMA concentration and cardiovascular disease. Elevated ADMA concentration has a high prevalence in hypercholesterolemia, hyperhomocysteinemia, diabetes mellitus, peripheral arterial occlusive disease, hypertension, chronic heart failure, coronary artery disease, pregnancy-induced hypertension and preeclampsia, erectile dysfunction, and other diseases. The observation made in the late 1990s that ADMA levels increased early during the development of atherosclerosis
suggested that ADMA had the potential to be not only a marker, but a mediator of vascular lesions.

Data has recently accumulated from a series of clinical studies which confirm the potential role of ADMA as a marker of cardiovascular risk. High ADMA levels were found to be associated with carotid artery intima-media-thickness in a study with 116 clinically healthy human subjects. Taking this observation further, another study performed in 90 hemodialysis patients reported that ADMA prospectively predicted the progression of intimal thickening during one year of follow-up. In a nested case-control study involving 150 middle-aged, non-smoking men, high ADMA levels were associated with a 3.9-fold elevated risk for acute coronary events.

**ADMA, a Prognostic Factor for Cardiovascular Risk and Mortality**

Several prospective clinical trials have added to the evidence supporting the relationship between ADMA and patient outcome. In one study, 225 hemodialysis patients were followed for almost 3 years, and ADMA was determined to be the strongest predictor of cardiovascular events and total mortality. Patients whose ADMA levels at the beginning of the study were within the highest quartile had a 3-fold higher risk of death of any cause than patients with ADMA levels below the median [2]. Another study investigated factors related to outcome of patients undergoing intensive care unit treatment for multiple causes. ADMA levels in the highest quartile were associated with a 17-fold excess in mortality as compared to patients with ADMA levels in the lowest quartile. In a third prospective study the outcome of patients with stable angina pectoris after percutaneous intervention was addressed, and again, patients with high ADMA levels were found to have a clearly elevated risk of developing severe cardiovascular complications. In all of these studies, other cardiovascular risk factors and confounding variables were included into the analyses, and ADMA was always found to predict cardiovascular risk independently of other variables. Thus, it has been concluded that ADMA is a novel cardiovascular risk factor [1,3,4].

Further prospective studies are currently under way to explore the role of ADMA for prediction of vascular disease and mortality in pulmonary hypertension, acute coronary syndrome, congestive heart failure, and in the general population. Updated information on ADMA can be found at www.allaboutadma.com [5].

**Methods to determine ADMA in human plasma or serum**

Quantification of ADMA by high performance-liquid chromatography (HPLC) has been the most widely applied method. HPLC analysis is usually performed after extraction of samples with cation-exchange columns followed by pre-column derivatization with o-phthalaldehyde followed by reversed phase HPLC with fluorescence detection. Several modifications of this method have been developed with respect to the extraction procedure, the derivatization reagents, or the HPLC columns used. Distinct from HPLC with fluorescence detection, other analytical strategies were applied, among them capillary electrophoresis, liquid chromatography – tandem mass spectrometry (LC-tandem MS), and gas chromatography – tandem mass spectrometry (GC-tandem MS). All of these methods are laborious, not readily available in many laboratories, and not applicable for routine diagnostic use.
We recently developed and validated an ELISA assay for the determination of ADMA levels in serum, plasma, or other biological fluids. The ADMA ELISA kit is based on the principles of a competitive immunoassay. In contrast to previously available methods for measuring ADMA, the new ELISA is easy to use and constitutes a high throughput technique. The combination of an acylation step and the competitive design of the ELISA resulted in a specific, highly sensitive, and non-isotopic immunoassay. The selected antiserum is specific for ADMA and results in negligible cross reactivities for L-arginine (< 0.02%) and other endogenous derivatives of L-arginine. The precision of our ELISA has been demonstrated by low intra- and interassay coefficients of variation (interassay, 8.3-10.3%; intraassay, 4.5-7.5%). ADMA concentrations can be accurately measured across the full range of physiologically relevant concentrations (i.e. 0.05 µmol/l to 2 µmol/l). The values derived from the ELISA correlate well with expected values in recovery tests (mean recovery from all serum samples was 94.6%), and they manifest excellent linearity in dilution studies. By comparison with LC-MS/MS, we found an excellent correlation (R = 0.984; p < 0.0001; [6]). The ELISA has been validated for human serum (which is the preferential matrix) and plasma, as well as for rat and mouse plasma and cell culture supernatants. It has thereby proven its suitability as a routine diagnostic tool in clinical chemistry as well as its applicability in experimental studies. The assay will contribute to improve our understanding of the role of ADMA in human health and disease.

References

5. All about ADMA. www.allaboutadma.com (last accessed on December 08, 2004).
### Table 1. Diseases that are associated with elevated ADMA levels.

<table>
<thead>
<tr>
<th>Condition</th>
<th>-fold increase vs. controls in case-control studies</th>
<th>increase in risk in prospective studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercholesterolemia</td>
<td>2-3</td>
<td>-</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Stable angina pectoris</td>
<td>2-3</td>
<td>3.9-fold</td>
</tr>
<tr>
<td>Acute coronary syndrome</td>
<td>3</td>
<td>yes</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>2-3</td>
<td>yes</td>
</tr>
<tr>
<td>Peripheral arterial disease</td>
<td>2-4</td>
<td>-</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>2-12</td>
<td>3-fold</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>2-3</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes mellitus type II</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>ICU treatment</td>
<td>-</td>
<td>17-fold</td>
</tr>
</tbody>
</table>

Data in row 2 indicate –fold increase in ADMA levels in the conditions specified in row 1, as assessed in cross-sectional studies. Data in row 3 indicate –fold increase in risk with elevated ADMA as compared to patients with low ADMA as assessed in prospective clinical studies.
Figure Legends

Figure 1.
Schematic representation of the pathophysiological sequelae of elevated ADMA levels, which result from its ability to inhibit endothelial NO formation.

Figure 2.
Relationship between the determination of ADMA in human serum by the novel ADMA-ELISA and by liquid chromatography – mass spectrometry.
Figure 2

Y = 0.85 * X + 0.011
r = 0.984
p < 0.0001
N = 29