ADMA: a mediator of endothelial dysfunction and marker of vascular disease

by Professor Rainer Böger

Asymmetric dimethylarginine (ADMA) is involved in the pathogenesis of hypertension and atherosclerosis through its inhibition of the formation of the endogenous vasculoprotective molecule, nitric oxide (NO). Determination of ADMA can thus help to predict both the likelihood of developing cardiovascular disease and its prognosis. A new competitive ELISA test for ADMA is a useful and fully validated tool suitable 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). NO is also involved in cell-cell interactions in blood vessels (e.g., inhibition of platelet adhesion and aggregation and inhibition of monocyte adhesion). As a result of these properties, NO has been described as an "endogenous anti-atherosclerotic molecule".

Dysfunction of the endothelial L-arginine/nitric oxide pathway is a common mechanism through which the deleterious effects of several cardiovascular risk factors on the vascular wall are mediated. Among such risk factors are hypercholesterolaemia, hypertension, smoking, diabetes mellitus, hyperhomocysteinaemia, and vascular inflammation.

ADMA, a mediator of endothelial dysfunction

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthesis, inhibiting vascular NO production within the concentration range found in patients with vascular disease. When infused intraarterially, ADMA also causes local vasoconstriction. The currently available experimental and clinical data suggest that even small changes in ADMA concentration can significantly alter vascular NO production, vascular tone, and systemic vascular resistance [1]. These properties justify the description of ADMA as a marker of endothelial dysfunction. Figure 1 illustrates schematically the pathophysiological roles of ADMA.

ADMA, a marker of vascular disease

To date, there are numerous studies that show a correlation between elevated ADMA concentration and cardiovascular disease. Elevated ADMA concentration is common in conditions such as hypercholesterolaemia, hyperhomocysteinaemia, diabetes mellitus, peripheral arterial occlusive disease, hypertension, as well as chronic heart failure, coronary artery disease, pregnancy-induced hypertension and pre-eclampsia, erectile dysfunction, and other diseases [Table 1]. In the late 1990s it was observed that ADMA levels increased at an early stage in the development of atherosclerosis giving rise to the possibility that ADMA is not only a marker but also a mediator of vascular lesions.

Data from a series of several recent clinical studies 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 of 116 clinically healthy human subjects. This observation was extended in another prospective study carried out in 90 haemodialysis patients where it was reported that ADMA could predict the progression of intimal thickening during one year of follow-up. In a nested case-control study involving 150 middle-aged, non-smoking males, high ADMA levels were found to be 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 correlation between ADMA and patient outcome. In one study, 225 haemodialysis patients were followed for almost 3 years. In this study, ADMA was found to be the strongest predictor of cardiovascular events and overall mortality. Patients whose ADMA levels at the beginning of the study were within the highest quartile had a 3-fold higher risk of death from any cause than patients with ADMA levels below the median.

Another study investigated the correlation between several factors and the outcome of patients who were being treated in intensive care units for a variety of conditions. ADMA levels in the highest quartile were associated with a 17-fold excess in mortality 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 investigated. Again, patients with high ADMA levels were found to have a clearly increased risk of developing severe cardiovascular complications.

In all of these studies, other cardiovascular risk factors and confounding variables were taken into account in the analyses. ADMA was always found to predict cardiovascular risk independently of such other variables. It has thus been concluded that ADMA is a novel cardiovascular risk factor [1, 2, 3].

Further prospective studies are currently under way to explore the role of ADMA in the prediction of vascular disease and mortality in pulmonary hypertension, acute coronary syndrome, congestive heart failure, and in the general population.

Methods for the determination of ADMA concentrations in human plasma or serum

High performance-liquid chromatography (HPLC) has been the most widely applied method for the quantification of ADMA in human plasma or serum. HPLC analysis is usually preceded by extraction of the samples using cation-exchange columns. The sample then undergoes pre-column derivatisation using o-phthalaldehyde followed by reversed phase HPLC with fluorescence detection. Several modifications of this method have been developed involving the extraction procedure, the derivatisation reagents, or the HPLC columns used.

In addition to such approaches using HPLC with fluorescence detection, other analytical techniques...
have been used. These include 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, frequently unavailable in many laboratories, and generally not applicable for routine diagnostic use.

We recently developed and validated an ADMA ELISA assay based on the principle of competitive immunoassay for the determination of ADMA levels in serum, plasma and other biological fluids. In contrast to the other methods for measuring ADMA, the new ELISA test is easy to carry out and can be used as a high throughput method. The combination of an acylation step and the competitive principle of the ELISA tests has resulted in a specific, highly sensitive and non isotopic immunoassay.

The antiserum used in the assay is specific for ADMA and shows negligible cross reactivities with L-arginine (<0.02%) and other endogenous derivatives of L-arginine. The high precision of the ELISA test can be seen from the low coefficients of variation (inter-assay C.V. 8.3 - 10.3%; intra-assay C.V. 4.5 - 7.5%). The ELISA assay can accurately measure ADMA concentrations over the full range of physiologically-relevant concentrations (i.e. 0.05 µmol/L to 2 µmol/L). The results of the ELISA test correlate well with expected values in recovery tests (mean recovery from all serum samples was 94.6%). In dilution studies, the ELISA data show excellent linearity.

An excellent correlation was found between LC-MS/MS and the ELISA test ($R = 0.984; p < 0.0001$) [Figure 2]. The ELISA test has been validated for both human serum (which is the preferred matrix) and plasma, as well as for rat and mouse plasma and cell culture supernatants. It has thereby shown its suitability as a routine diagnostic tool in clinical chemistry as well as its applicability to experimental studies. The ELISA assay has great potential for improving our understanding of the role of ADMA in human health and disease.

Table 1. Diseases that are associated with elevated ADMA levels. 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.

<table>
<thead>
<tr>
<th>Condition</th>
<th>-fold increase vs. controls in case-control studies</th>
<th>-fold 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>2-3</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>

References

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