How to Evaluate Endothelial Function?

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Normal Endothelium

Endothelium-Dependent Stimuli

- NO↑, A-II↓, ET-1↓

Vasodilatation

In patients with risk factors for coronary disease or with established atherosclerosis

- NO↓, A-II↑, ET-1↑

Endothelial dysfunction becomes apparent by decreased or paradoxical responses
How to Evaluate?

1. Invasive versus Noninvasive

2. With what?
   - Ach
   - Flow
   - Cold Pressor

3. Where?
   - Conduit Vessel
   - Resistance Vessel

4. Accuracy? Gold standard?

5. Correlation between peripheral and coronary circulations?
Endothelium-Dependent Vasodilator

Acetylcholine (Ach)

- Endothelial-mediated Dilation
- Smooth muscle-mediated Constriction
Assessment of endothelial vasomotion using Acetylcholine

Before Ach

After Ach
Assessment of Endothelial Function of Coronary Resistance Vessels using Intracoronary Doppler Techniques

Baseline
Acetylcholine

Normal endothelial function of resistance arterioles
Evidence of Impaired Endothelium-Dependent Coronary Vasodilatation in Patients with Angina Pectoris and Normal Coronary Angiograms

The loss of endothelium-dependent dilation occurs early in atherosclerosis, even prior to its detection by angiography.

Coronary Endothelial Dysfunction After Heart Transplantation Predicts Allograft Vasculopathy and Cardiac Death

Change in epicardial coronary artery area in response to acetylcholine

11.1 ± 2.9%

Change in microvascular coronary artery flow in response to acetylcholine

75 ± 20%

# Methods used for *in vivo* endothelial function testing

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<th>Vascular bed</th>
<th>Invasiveness</th>
<th>Accuracy/reproducibility</th>
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<td>Venous occlusion plethysmography (VOP)</td>
<td>Forearm resistance vessels</td>
<td>Moderately invasive</td>
<td>Highly accurate and reproducible. Regarded by some as the ‘Gold Standard’</td>
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<tr>
<td>Flow-mediated dilatation (FMD)</td>
<td>Brachial (conduit) artery</td>
<td>Non-invasive</td>
<td>Accuracy depends on quality of equipment/software. Observer variations</td>
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<td>Laser Doppler iontophoresis</td>
<td>Forearm skin microvessels</td>
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<td>Pulse wave analysis</td>
<td>‘Global’ endothelial function</td>
<td>Non-invasive</td>
<td>Unclear how accurately global arterial stiffness reflects endothelial function</td>
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Venous Occlusion Plethysmography (VOP)

- Function of Resistance vessel in the forearm
- Assessed by strain-gauge venous impedance plethysmography
- Examines the change in forearm blood flow in response to direct intraarterial (brachial artery) administration of agonists
Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension.

Brachial Artery Flow-mediated Dilatation

Shear stress

Endothelial cell

O₂⁻

OOONO⁻

Vascular smooth muscle cell

Relaxation

GTP → cGMP

Ca²⁺ → L-Citrulline

Calmodulin

NADPH

BH₄

L-Arginine

eNOS

GC⁺
Shear stress-induced NO release and subsequent vasodilatation (Flow-Mediated Dilatation, FMD)

Baseline Flow
BA Diameter

250 mmHg for 4.5-5 min

60 sec after cuff release

Reactive Hyperemia Flow
BA Diameter

4.20mm

4.88mm

%FMD=14%
Major limitations
1. Need for ultrasonographic expertise
2. Significant day-to-day variability (about 25%) due to biological circadian rhythms.
Iontophoresis in Conjunction with Laser Doppler Imaging

Laptop computer controls current delivery from MIC1-e and records perfusion data from DRT4 (Moorsoft for Windows).

Key:
- Positive current
- Negative current
- Fibre-optic laser probes

* Anode and cathode are placed on forearm and filled with SNP and ACh respectively.
Non-invasive assessment of endothelial function-relation between vasodilator responses in skin microcirculation and brachial artery

Skin microvascular perfusion

Augmentation Index

Radial artery waveforms

Summary of the multiple causes and locations of arterial stiffness

Endothelial Cells:
- Endothelial dysfunction
- Increased permeability

Intima:
- ↑ Collagen, AGE’s, MØ
- Leukocyte, I-CAM
- MMP, TGF-β, VSMC
- ↓ Elastin

Media
- ↑ VSMC, Collagen, AGE’s, MMP
- ↓ Elastin

Adventitia
- ↑ Collagen, Fibroblast

Extrinsic influences:
- NaCl, lipid, angiotensin, Sympathetic Neurohormone, shear stress, increased luminal diameter

Factor contributing to increase arterial stiffness

- Dyslipidemia
- Hyperinsulinemia
- Hyperglycemia
- Hypertension
- Endothelial Dysfunction

- Oxidative Stress
- Aging

- Elastin fracture
  - ↑ Arterial Stiffness
  - ↑ Collagen deposition

- Left ventricular Dysfunction/Hypertrophy
  - ↑ Pulse pressure/Hypertension
  - Myocardial ischemia/↑ Metabolic demand

Stiffness
Relationship between Pulse pressure and Atherosclerosis

- ↑ PP
- Vascular endothelial damage & mechanical fatigue
- ↑ Central wave reflection
- Atherosclerosis
- ↑ Aortic stiffness
C2, small artery elasticity; C1, large artery elasticity; Pres: Vol, pressure/volume relationship of a single artery; Exer BP, blood pressure rise in response to programmed exercise test; PWV, pulse wave velocity; Pulse Pres, auscultatory pulse pressure.
Reduced small artery but not large artery elasticity is an independent risk marker for cardiovascular events.


Reduced small artery elasticity, which is a measure of endothelial dysfunction, is significantly associated with cardiovascular events independent of age.
Reduced arterial elasticity is associated with endothelial dysfunction in persons of advancing age: Comparative study of noninvasive pulse wave analysis and laser Doppler blood flow measurement

Assessment of peripheral vascular endothelial function with finger arterial Pulse Wave Amplitude

: Finger plethysmograph (Peripheral arterial tonometry [PAT])

Jeffrey T et al. Am Heart Journal 2003;146:1684

\[ r = 0.55, \quad P < 0.0001 \]
Relationship Between Carotid Artery Intima-Media Thickness and Brachial Artery Flow-Mediated Dilation in Middle-Aged Healthy Men

n = 1,578
r = -0.006, p = 0.82

Interrelations Between Brachial Endothelial Function and Carotid IMT in Young Adults.

The Cardiovascular Risk in Young Finns Study

Comparison of IMT in SLE patients and healthy controls

Shin YJ & Youn HJ. 2005 (unpublished data)
Relationships between FMD and IMT in patients with SLE

N=202

$r = -0.254$, $P=0.016$

Shin YJ & Youn HJ. 2005 (unpublished data)
Flow Improvement after Adenosine infusion: Study using Transthoracic Doppler Echocardiography

Baseline

Hyperemia

![Baseline Image]

![Hyperemia Image]

**CFR** = \[
\frac{\text{Hyperemic PDV}}{\text{Baseline PDV}}
\]

Baseline PDV = 22.6

Hyperemic PDV = 56.2

CFR = 2.5
Endothelial Release of Nitric Oxide Contributes to the Vasodilator Effect of Adenosine in Humans

1. Both endothelium-independent and endothelium-dependent dilation; at high concentration of adenosine, endothelium independent dilation dominates while NO contributes to the dilator effects of adenosine at lower adenosine concentration.
2. Triggered nonspecifically by the increase in flow (shear stress) rather than by the specific stimulation of endothelial adenosine receptors.

Peripheral vascular endothelial function is impaired in patients with microvascular angina.

Penetrating Intramyocardial Coronary Artery (PICA)

Adenosine
Measurement of PICA-CFR

Adenosine 140 µg/kg/min

Baseline

Hyperemia

Width of color signals

Width Ratio

Pulsed Doppler signals

PDV

PICA-CFR

Inhibition of Nitric Oxide Synthesis During Cold Pressor Test in Patients With Coronary Artery Disease

Tousoulis D et al. Am J Cardiol 1997;79:1676
Changes of Coronary Artery Flow During Cold Pressor Test using Transthoracic Doppler Echocardiography
Is endothelial function testing ready for “prime time” clinical use?