Endothelial function testing

Martin A. Ritter  
Dept. of Neurology  
University of Münster, Germany
Why testing?

• Atherosclerosis begins early (adolescence)
• Endothelium is
  – Key regulator of vascular homeostasis
    • Barrier function **AND**
    • signal transducer
• Endothelium function (EF) very sensitive to stressors
• Alteration in EF **precedes** morphological atherosclerotic changes
Tests in atherosclerosis

EF testing  Carotid IMT  Duplexsono
Augmentation index and Pulse wave velocity
MRI (angiography)  CT (angiography)

### Available tests of Endothelium

<table>
<thead>
<tr>
<th>Technique (Outcome Measure)</th>
<th>Noninvasive</th>
<th>Repeatable</th>
<th>Reproducible*</th>
<th>Reflects Biology</th>
<th>Reversible</th>
<th>Predicts Outcome†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac catheterization (change in diameter, change in coronary blood flow)</td>
<td>−</td>
<td>−</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Venous occlusion plethysmography (change in forearm blood flow)</td>
<td>−</td>
<td>+/−</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Ultrasound FMD (change in brachial artery diameter)</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PWA (change in augmentation index)</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PCA (change in reflective index)</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>PAT (change in pulse amplitude)</td>
<td>+</td>
<td>+</td>
<td>+/−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

+ supportive evidence; −, insufficient evidence;

FMD, flow-mediated dilatation; PWA, pulse wave analysis; PCA, pulse contour analysis; and PAT, pulse amplitude tonometry.

* Reproducibility of PWA, PCA, and PAT less extensively investigated than FMD.
No studies link PWA, PCA, and PAT to outcome

Deanfield 2007
Other available tests

- Invasive techniques
  - Intrabrachial infusion of vasoactive agents

- Non-invasive techniques
  - Cold pressor stress (CPT)
  - Isometric hand grip exercise
  - Assessment of endothelium independent vasodilation
Intracoronary infusions

- Drug infusions via cardiac catheter for 2-3 min
- Coronary blood flow = velocity x cross-sectional area
  - Acetylcholine (Ach) at increasing rates (e.g. 1-10 µg/min)
  - Intact endothelium → dose dependent dilation
  - Endothelial dysfunction → decreased vasodilatory response or even vasoconstriction
- Gold standard but
  - Invasive
  - No screening
  - Difficult to reproduce
Ach – infusion and long term survival

Intracoronary acetylcholine infusion in 308 patients

Halcox 2002
Intrabrachial infusions

• Arterial catheter into the brachial (femoral) artery
• Forearm blood flow measured by plethysmography
  – ACh, bradykinin, phenylephrine, L-arginine etc.
  – nitroprusside infusion: endothelium independent dilatation?
• Limitations as with intracoronary infusions
Non-invasive techniques - Cold pressor stress test (CPT)

- Endothelium dependent vasodilation by release of catecholamines
- One hand in ice water for 2 min
- Determination of
  - coronary blood flow (invasive)
  - or diameter of the brachial artery by US
Non-invasive techniques – Carotid reactivity to isometric exercise

- Static isometric hand-grip exercise (IHG)
  - sustain a hand grip at 33% of peak effort for 2 min
- changes in the carotid (brachial) artery diameter by US
- baseline and every 30 s interval during IHG for 10 min.
Non-invasive techniques - Flow mediated (vaso)-dilation (FMD)

- Dilation of arteries induced by increased flow (mostly: A. brachialis)
- Background:
  - Ischemia – postischemic hyperemia – increase of flow - increased flow velocity – increased shear stress
  - Shear stress – NO-production ↑ - dilation
- FMD of brachial artery
  - baseline Ø ~3-4 mm
  - Postischemic increase by ~10%

Celermajer 1992
**Endothelium-independent** vasodilation with nitroglycerin (NTG)

- After FMD 10 min of waiting time
- NTG (0.4mg sublingual) (NO-donor)
- Max. vasodilation 3 to 4 min. after NTG
- Continuous imaging
- In most studies → little effect of diseases on this response
FMD technique

Endothelium independent

0.4 mg NTG
Measure after 4 min.

Even in patients at risk normal!

FMD is reported as max. % change from baseline
Normal: ~10%

Ischemia (RR-cuff 30 mmHg > syst. RR)
Placement of cuff variable
Doppler sonography of brachial artery (>7.5 MHz, B-mode)
Determination of baseline diameter
Sonography of brachial artery for at least 90s
Max. dilation at 50-60s after cuff release (postschleial dilatation) velocity (angio correction)
FMD: Example

Baseline 60 s after cuff release

FMD = \frac{(0.35-0.31)}{0.35} \times 100 = 11\%
FMD of the brachial artery
FMD of the brachial artery
FMD - plausability

• Low nitrate levels correlate with reduced FMD (Kleinbongard 2006)
• Allows risk estimation for future coronary events (Schachinger 2000)
• Correlates with risk factors (Smoking, RR, Cholesterol, Age) (Woo 2004, Faulx 2003)
FMD - plausability

Risk Factor Score
Cholesterol, smoking, mean blood pressure, family history, age, gender.

Woo 2004
FMD - plausability

• Correlates with manifest vascular disease
  – ICA-Stenosis (Hsu 2005)
  – PAD (Hu 2000)

• Interventions increase FMD
  – Laughing, Chocolate, Wine, medication, life-style modification (Faulx 2003)
The good news for X-mas: Chocolate protects !?!
Laughter helps blood vessels function better!

Lachen ist gesund:
Lachen verbessert die Endothelfunktion und beugt einer Atherosklerose vor.


 Bereits in einer früheren Studie konnte Michael Miller von der Universität von Maryland in Baltimore zeigen, dass humorvolle Menschen seltener an einem Herzinfarkt erkranken als strebsame Karrieremenschen. In der aktuellen Studie wurden diese Ergebnisse durch eine Prüfung der Endothelfunktion, bezeichnet als "flow mediated dilation" (FMD), nochmal bestätigt.
FMD and cardiovascular outcome
Flow mediated dilatation changes after 6 mo antihypertensive therapy

It’s reversible!
FMD – IMT

• FMD correlates with IMT (p < 0.0001)
  – Adjusted for risk factors
  – 2100 healthy people 24-39 years

  Juonala 2004

• FMD correlates with blood pressure (p=0.01)
  – But not with IMT (!)
  – 1580 healthy men 40-60 years

  Yan 2005
FMD - issues

• Technically challenging
  – 200 investigations recommended (Corretti 2003)

• Methodological inconsistencies of studies (Bots 2005)
  – population (children - elderly, healthy - ill)
  – timing (morning, noon or evening)
  – Where to measure (upper arm - forearm)
  – Duration of ischemia (3 min., 4 min., 5 min.)
  – Where to place the cuff (upper arm - forearm)
  – When to measure after cuff release (40s, 60s, 90s)
Correlates only for very low (<3%) 10-year risk of vascular event
No correlation with higher risk scores
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<th>FMD- issues</th>
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<tr>
<td>• Studies show inconsistent prognostic value</td>
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<tr>
<td>• Intervention studies positive but very restricted</td>
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<tr>
<td>- Technically challenging</td>
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<tr>
<td>- Methods vary</td>
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<tr>
<td>• NO study demonstrates</td>
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<td>- FMD improvement = reduction of endpoints</td>
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FMD needs further evaluation
Conclusions

• Endothelial dysfunction is systemic and prognostically relevant
• FMD testing is the best evaluated non-invasive test of endothelium
• Not yet suitable for screening / individual decision-making
• Associated with disease burden and outcome
• „Probable valid biomarker“
• Improvement in endothelial function = improved outcome?
Thank you!