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HRVATSKO DRUŠTVO ZA
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New biomarkers of endothelial function

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Traditional assessment of endothelial function

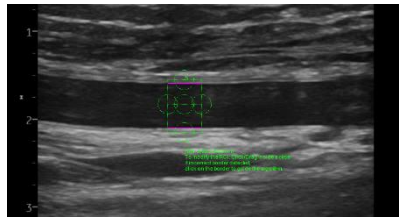
Assessment of endothelial function has traditionally been based on endothelium-dependent vasomotion

It can be measured in the coronary or peripheral circulation

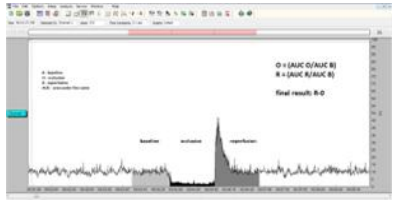
Coronary endothelial function is most commonly assessed by **invasive methods**, using angiography and the vasodilatory response to **acetylcholine** or **nitroglycerin**.
→ **Limitations:** invasiveness, operator dependence, high cost, and low availability.

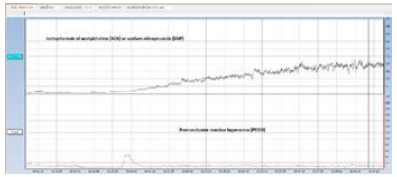
Peripheral noninvasive methods are now widely used since endothelial dysfunction is a **systemic condition**

Flow-mediated dilation (FMD) – the most commonly used method:
- Measures the ability of arteries to release **nitric oxide (NO)** in response to **reactive hyperemia**.
- **Advantages:** low cost, prognostic value for cardiovascular disease.
- **Limitations:** dependent on cardiac output, provides limited quantitative data, and is operator-dependent.



New noninvasive methods:
- **Peripheral arterial tonometry (PAT)**
- **Laser Doppler flowmetry (LDF)**





New biomarkers of endothelial function

Blood endothelial biomarkers have emerged as important alternatives to traditional methods for early diagnosis and cardiovascular risk stratification.

They offer a potentially more reliable, specific, comprehensive, simple, and inexpensive way to assess endothelial (dys)function.

Characteristics of an ideal endothelial biomarker:

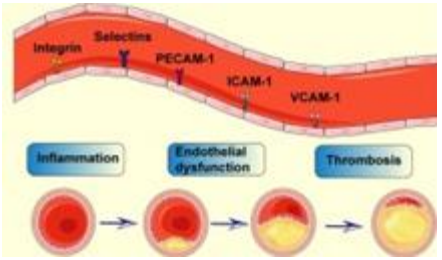
- Endothelium-specific and representative of the underlying disease
- Reproducible and useful for clinical evaluation in a single measurement
- Correlated with disease severity
- Quantifiable by simple and low-cost methods

Because the endothelium has multiple functions, a wide panel of biomarkers has been explored, reflecting dysfunction in various endothelial properties.

Most studied biomarkers include:
Endothelial progenitor cells (EPCs)
Endothelial microparticles (EMPs)
MicroRNAs (miRNAs)
Adhesion molecules

→ Despite their potential, **standardized assays and protocols** for assessing endothelial damage are **not yet available** and remain **restricted to clinical research**.

Classic circulating markers of endothelial dysfunction (ED)



- **Adhesion molecules**:

  E-selectin, ICAM-1, VCAM-1

  → Reflect endothelial activation and leukocyte adhesion

- **Coagulation-related markers**:

  von Willebrand factor (vWF) and **soluble thrombomodulin**

  → Indicate endothelial injury and prothrombotic state

Clinical relevance:

•Several markers have been evaluated for disease severity and prognosis in **sepsis**

•In **diabetes mellitus**, **chronic kidney disease**, **peripheral artery disease**, and **heart failure**,

- Classic biomarkers correlate with disease severity <u>but their **predictive value for cardiovascular risk** remains debatable</u>.

Limitations:

•Many of these molecules are produced by **leukocytes and platelets**, not exclusively by the endothelium.

•They **do not reliably reflect coronary endothelial function**.

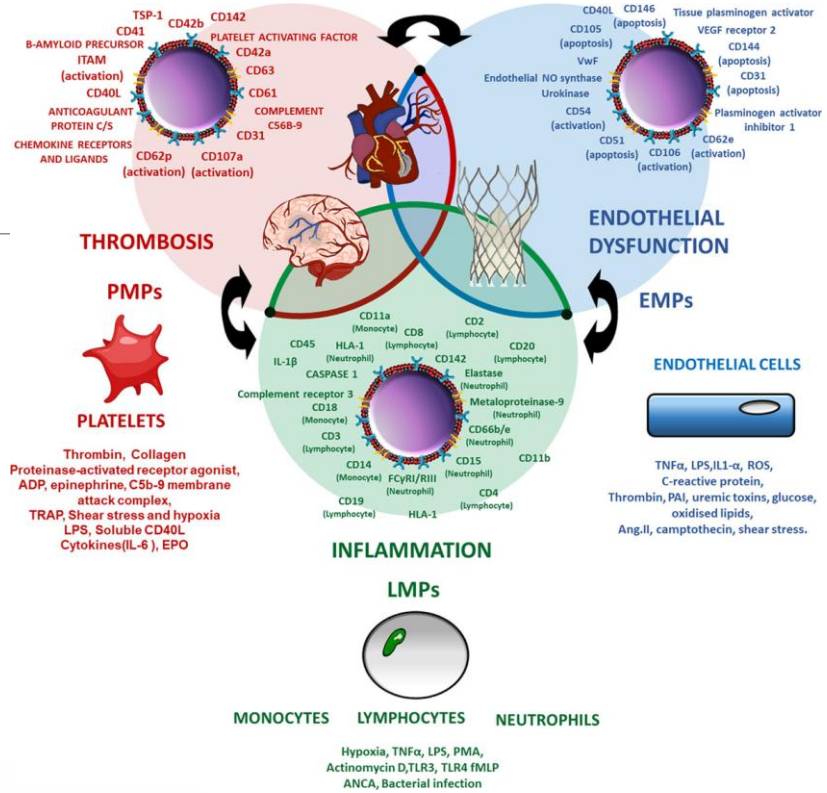
•Among classical markers, **vWF** remains the most consistent predictor of **cardio- and cerebrovascular events** in healthy, hypertensive, hyperlipidemic, and cardiac patients.

Source: Lugo-Gavidia et al. Hypertension 2021. https://orcid.org/0000-0002-0052-8388

Microparticles (MPs) / Extracellular vesicles (EVs): Origin and Function

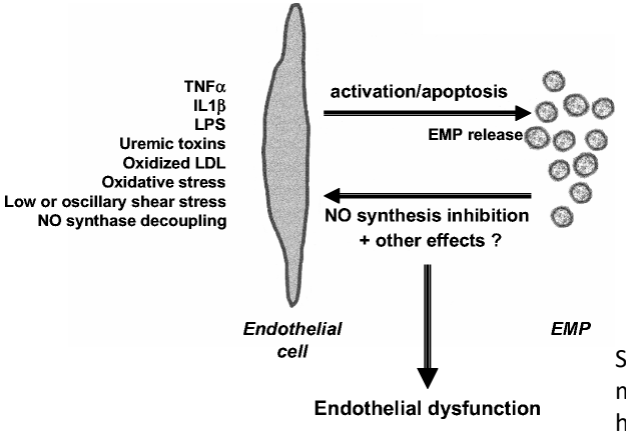
Microparticles (MPs) - small membrane vesicles (0.1–1.0 μm) released from **platelets, leukocytes, or endothelial cells (ECs).**

- Platelet MPs - the most abundant
- MPs carry adhesion molecules, pro-/anticoagulant factors, and microRNAs, transferring biological information between cells and influencing gene expression and cell signaling



[Endothelial MPs (EMPs)](#) are shed when the endothelium is **activated or injured**.

- Their release is stimulated by **TNF-α, thrombin, uremic toxins, PAI-1**, and **reactive oxygen species (ROS)**.



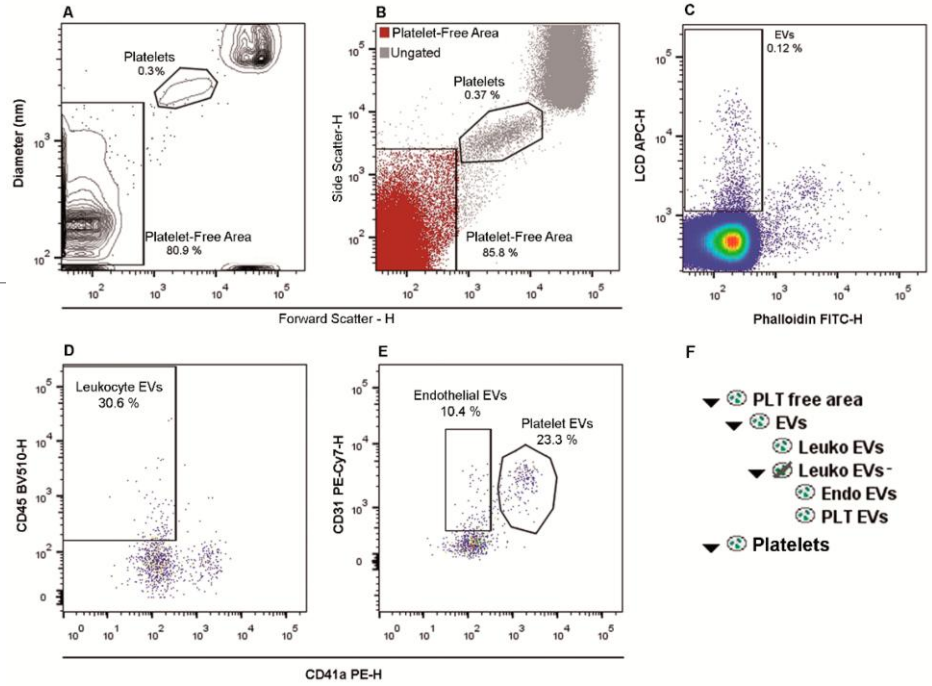
Source: Chironi, G.N., Boulanger, C.M., Simon, A. et al. Endothelial microparticles in diseases. Cell Tissue Res 335, 143–151 (2009). https://doi.org/10.1007/s00441-008-0710-9

Microparticles (MPs) / Extracellular vesicles (EVs): Quantification

Flow cytometry – the most widely used technique; identifies MPs by **size and surface markers** indicating the cell of origin.

- Other methods: **atomic force microscopy (AFM)** and **nanoparticle tracking analysis (NTA)** for smaller vesicles; **electron microscopy** for biochemical and morphological characterization.

- **Healthy individuals:** ~10^3–10^4 EMPs/mL plasma
- **Cardiovascular, metabolic, and autoimmune diseases:** ≥10× increase due to endothelial stress
- Results can be affected by **lipid levels** and **instrument sensitivity** (detection limit ~0.5 µm)



Gating Strategy for extracellular vesicles (EVs) identification and subtyping.
Source: Int. J. Mol. Sci. 2021, 22(1), 48;https://doi.org/10.3390/ijms22010048

Challenges and Perspectives

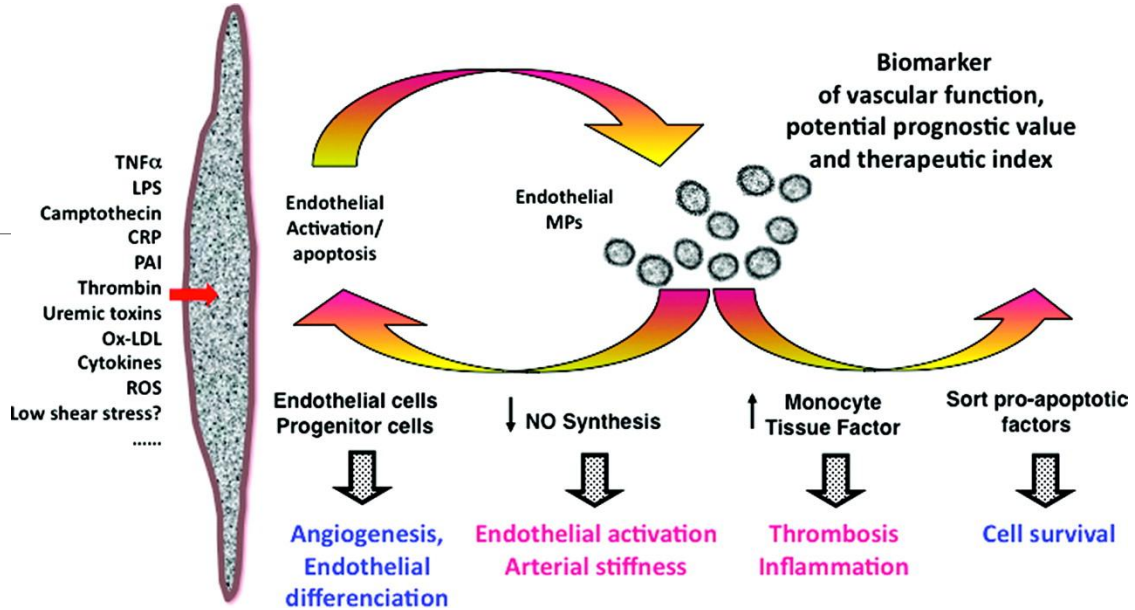
- High methodological variability limits comparability across studies.
- Standardization of detection and quantification protocols is essential before clinical application.

Microparticle Actions on the Endothelium

Microparticles (MPs) are key mediators of intercellular communication in both physiological and pathological states.

They modulate cellular processes such as: angiogenesis, inflammation, endothelial activation and coagulation

Determinants of MP effects: cell of origin, concentration, releasing stimulus, underlying disease



Source: Dignat-George F, et al. The many faces of endothelial microparticles. Arterioscler Thromb Vasc Biol. 2011. PMID: 21160065 Review.

- **In vitro and in vivo studies** show that endothelial and non-endothelial MPs from pathological conditions can:
  - Impair **NO-dependent vasodilation**
  - Increase **endothelial permeability** and **oxidative stress**
  - Promote **release of pro-inflammatory cytokines**
  - Induce **EC apoptosis** and impair **angiogenesis**
- **Procoagulant e**ndothelial MPs found to be elevated in diseases such as **type 2 diabetes** and **coronary artery disease (CAD)**.

Protective effects:

•Depending on their **composition and context**, MPs may support **vascular regeneration and repair**.

•Positive effects on **post-ischemic neovascularization** have been demonstrated in experimental studies.

Microparticles as Biomarkers of Endothelial Dysfunction (ED)

Endothelial microparticles (EMPs) are closely associated with endothelial function and reflect ongoing pathogenic processes.

Increased EMP levels have been observed in the presence of:

Cardiovascular risk factors: smoking, obesity

Cardiovascular and metabolic diseases: coronary artery disease (CAD), diabetes mellitus, metabolic syndrome, stroke, pulmonary hypertension, renal failure, heart failure

Rheumatic diseases with strong vascular involvement due to autoimmunity, chronic inflammation, and accelerated atherosclerosis

Table 1. Quantification of EMPs in Diseases.

Disease/ Condition	EMP Phenotype								Major Findings	References
	CD31	CD51	CD54	CD62E	CD105	CD144	CD146	AnnV		
Smoking						↑			Active smoking caused a significant increase in the number of EMPs	Mobarrez et al[70]
Arterial hypertension	↑								EMPs are strongly correlated with the level of both systolic and diastolic blood pressures	Preston et al[77]
Pulmonary hypertension	↑			↑		↑			$CD31^+$ and $CD144^+$ EMPs predict the hemodynamic severity of the disease (measured by right heart catheterization)	Amabile et al[53]
End-stage renal disease	↑						↑		EMPs are highly correlated with impaired vascular function (measured by FMD) and indices reflecting arterial stiffness	Amabile et al[62]
	↑						↑		EMPs are inversely correlated with shear stress and apoptosis	Boulanger et al[78]
Obesity	↑								EMP levels are negatively correlated with FMD	Esposito et al[71]
	∅	↑				∅			$CD51^+$ EMPs are increased in obese patients with hypertension, but not in nonobese hypertensive patients; $CD31^+$ only differentiated hypertensive patients from healthy controls	Hu et al[79]
MS							↑		EMP levels are associated with the evolution of the disease	Agouni et al[80]
						↑			EMPs are correlated with individual metabolic abnormalities and oxidative stress	Helal et al[81]

Source: Leite AR. Angiology. 2020 May;71(5):397-410. doi: 10.1177/0003319720903586.

Clinical relevance:

EMPs can be useful for CV risk stratification

Monitor disease activity and severity

Assess effectiveness of atheroprotective treatments

Advantages over other biomarkers:

More stable than NO, eNOS, or ET-1

Less daily fluctuation

Not influenced by pharmacologic agents (e.g., nitrates)

Endoglin (CD105): Origin and General Functions

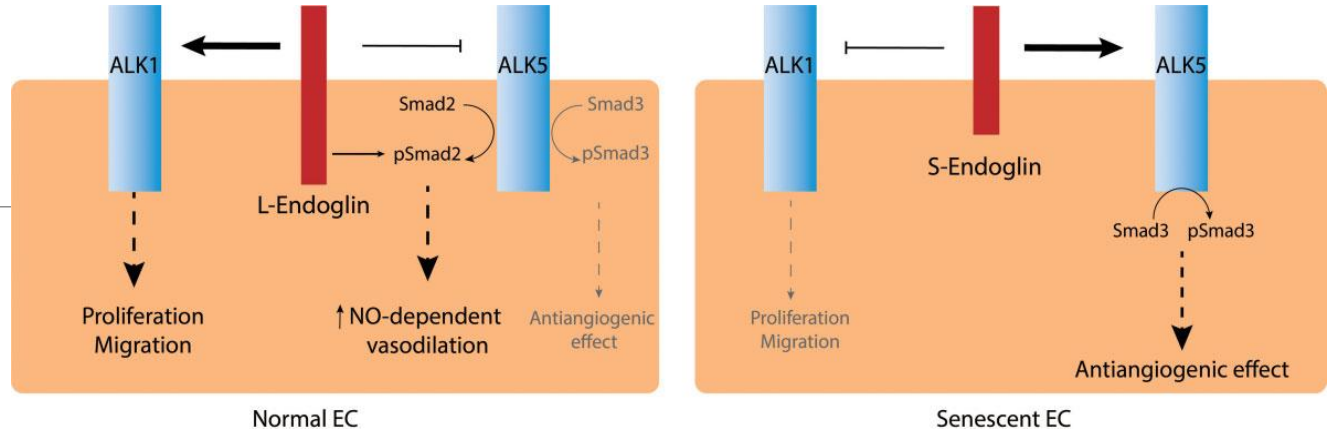
Endoglin (CD105) is a transmembrane receptor for TGF-β1 and TGF-β3, mainly expressed in proliferating endothelial cells (ECs).

Acts as a coreceptor, modulating TGF-β signaling.

TGF-β is essential for:

- Angiogenesis
- Cardiac and vascular development
- Vascular homeostasis

Defects in TGF-β/endoglin pathways lead to vascular abnormalities, e.g., hereditary hemorrhagic telangiectasia.



Long-form endoglin is present in normal ECs and stimulates angiogenesis via the TGF-b/ALK1 pathway, as well as NO-dependent vasodilation, via increasing Smad2 protein levels. On the other hand, L-endoglin inhibits TGF-b/ALK5/Smad3 signaling.

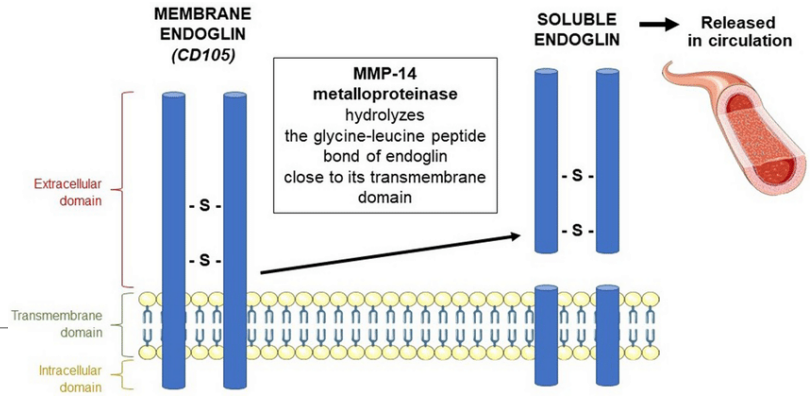
S-endoglin is present in senescent ECs and has an antiangiogenic effect, by stimulating the TGF-b/ALK5/Smad3 pathway. This molecule also inhibits the TGF-b/ALK1 pathway.

L-endoglin has a proangiogenic role and S-endoglin has an antiangiogenic role.

L-endoglin increases the levels of Smad2 protein, improving vasodilation. S-endoglin impairs NO-dependent vasodilation, although the molecular mechanisms involved are not yet well understood.

Source: Leite AR. Angiology. 2020 May;71(5):397-410. doi: 10.1177/0003319720903586.

Endoglin, TGF-β Signaling Pathways, and Atherosclerosis



Source: Margioula-Siarkou et al. Molecular and Cellular Biochemistry. 477. 10.1007/s11010-021-04294-z.

Complex effects of TGF-β in atherogenesis:

- Promotes **stable atherosclerotic lesions**
- Stimulates extracellular matrix synthesis and deposition
- **Suppresses inflammation and ROS production**
- **Inhibits VSMC proliferation and migration**

Endoglin expression in plaques:

- Both **L-endoglin** and **S-endoglin** are upregulated in **atherosclerotic plaques**
  - **L-endoglin:** preserves endothelium, regulates vascular tone, proangiogenic
  - **S-endoglin:** antiangiogenic, opposes L-endoglin, impairs NO-dependent vasodilation

Soluble endoglin (sEng)

• Generated by **cleavage of membrane endoglin** by metalloprotease MT1-MMP
• Detected in **plasma and urine** (ELISA and Western blot)

• Released in response to **hypoxia, inflammation, oxidative stress, and proatherogenic mediators**
• Acts as a **scavenger of TGF-β ligands**, inhibiting TGF-β signaling and eNOS expression
• Contributes to atherogenesis, endothelial inflammation, and impaired vasodilation
• Clinical observations:
  - sEng reduces active TGF-β levels in atherosclerosis
  - Low serum TGF-β1 is a **risk factor for atherosclerosis** in high-risk patients (e.g., end-stage renal disease)

• sEng mechanisms in endothelial dysfunction are **not fully understood**, but it is a promising **biomarker and potential therapeutic target** in atherosclerotic disease

Soluble Endoglin (sEng) as a Biomarker of Endothelial Dysfunction

- **sEng levels** are elevated in patients with **atherosclerosis**, especially **peripheral artery disease**, and correlate with **total cholesterol** *(Blann et al. Atherosclerosis 1996.)*
- **Plasma sEng increases** in response to:
  - **Hypercholesterolemia**
  - **Arterial hypertension**
  - **Diabetes mellitus**
  - **Inflammatory states** *(Blazquez-Madela AM et al. BMC Med 2010.)*

Heterogeneity in sEng levels:

- sEng may **decrease as disease progresses**, reflecting complex regulation and formation of **sEng/TGF-β1 complexes** *(Li et al. Atherosclerosis 2010.)*
- Early vascular alterations may show **higher sEng**, whereas **advanced disease or acute myocardial infarction (AMI)** may show **lower levels**.

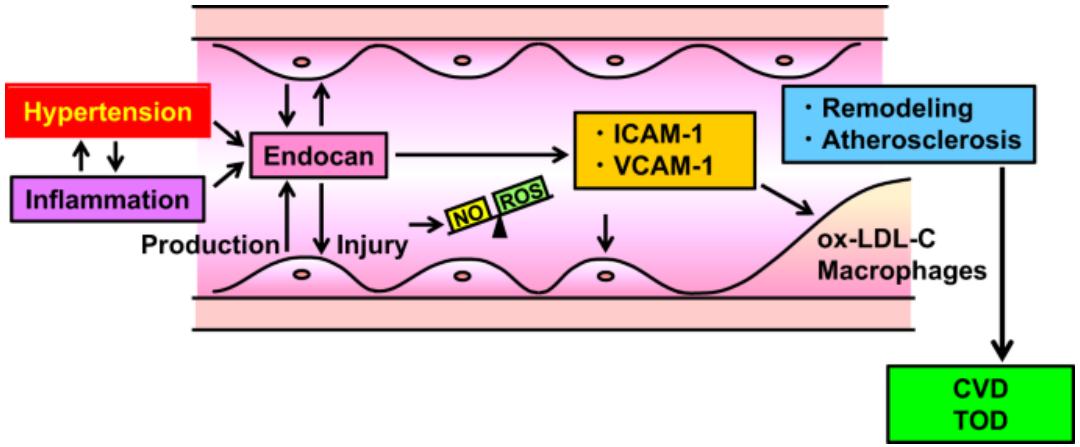
Clinical observations:

- **Early CAD / chest pain:** higher sEng than controls
- **Significant CAD or AMI:** lower sEng, associated with **unstable plaques** and **higher CV risk**
- **Stable CAD:** higher sEng predicts **future CV events**, especially **chronic heart failure** *(Li et al. Atherosclerosis 2010.)*
- **Heart failure with reduced ejection fraction:** higher sEng correlates with:
  - Increased **LV end-diastolic pressure**
  - Reduced **LV ejection fraction**
  - Worse **NYHA class** *(Kapur et al. A J Cardiol 2010.)*

- sEng is a **potential marker of endothelial injury, activation, inflammation, and senescence**.
- **Limitations:** variable results across studies; predictive value and reproducibility need further validation.

Endocan (endothelial cell-specific molecule-1, ESM-1)

- Soluble dermatan sulfate proteoglycan
- Secreted by **vascular endothelial cells (ECs)** and **epithelial cells** in renal distal tubules, bronchi, and lung submucosal glands
- Upregulated by **inflammatory stimuli** (e.g., lipopolysaccharide) and **cytokines** (TNF-α, IL-1β)
- Participates in several vascular processes regulating endothelial activation, permeability, and proliferation
- Enhances the production of pro-inflammatory cytokines by ECs, the expression of adhesion molecules (ICAM-1 and VCAM-1), and the adhesion between monocytes and ECs



Source: Hirooka, Y. Hypertens Res 47, 794–795 (2024). https://doi.org/10.1038/s41440-023-01542-1

Endocan as a Biomarker of Endothelial Dysfunction (ED)

- **Immunoinflammatory marker** of endothelial activation and dysfunction
- **Increased serum endocan levels** are observed in:
  - **Autoimmune and systemic inflammatory diseases:** Behçet disease, psoriasis, systemic sclerosis, systemic lupus erythematosus, sepsis, ARDS
  - **Cardiovascular/metabolic diseases:** hypertension, coronary artery disease (CAD), slow coronary flow, diabetes mellitus, chronic kidney disease, obstructive sleep apnea
  - **Other conditions:** hypothyroidism

Table 2. Endocan Levels in Vasculopathic Diseases.

Study	Year	Condition	Major Findings
Kose et al[redacted]	2015	Obstructive CAD	Increased endocan levels in patients with CAD No association is found between endocan levels and CAD severity (*Gensini* score) Positive correlation with number of diseased epicardial vessels Higher endocan levels in patients with diabetes and CAD
Çimen at al[144]	2016	Obstructive CAD and MVA	Endocan is an independent predictor of CAD Increased endocan serum levels in patients with MVA Positive correlation with severity of CAD (*Gensini* score)
Kundi et al[145]	2017	STEMI	Higher endocan levels are associated with a higher risk of STEMI
Wang et al[15]	2015	Hypertension	Strong correlation between endocan levels and cIMT Endocan is an independent predictor of the presence and severity of CAD
Musialowska et al[146]	2018	Hypertension	Strong correlation between endocan levels and cIMT
Ağaç et al[153]	2019	Hypertension	Endocan levels are not significantly different between patients with asymptomatic hypertension with or without target organ damage
Yilmaz et al[149]	2014	CKD	Increased endocan levels in patients with CKD Independent association with FMD and cIMT Independent predictor of mortality and CVE Better discriminative power for predicting CVE than cIMT and FMD

Clinical relevance:

- Correlates with **disease severity**, **FMD**, and **carotid intima–media thickness (cIMT)**
- Reflects **atherosclerotic burden** in psoriasis and lupus
- In **chronic kidney disease**, independently predicts **mortality and CV events**; potentially more accurate than FMD or cIMT
- Serial measurements can predict **outcomes in severe sepsis**

Advantages for clinical use:

- **High reproducibility, feasibility, and low cost** (ELISA kits)
- Suitable for **clinical implementation** as a biomarker of ED

Endocan is a **robust, versatile, and practical biomarker of endothelial dysfunction** across multiple cardiovascular and inflammatory conditions

Source: Leite AR. Angiology. 2020 May;71(5):397-410. doi: 10.1177/0003319720903586.

🎯 Conclusions

✔ The **endothelium** is a key **trigger and mediator** in many cardiovascular and systemic diseases.

✔ Developing **reliable techniques** to assess endothelial integrity is clinically important.

✔ **Blood biomarkers** offer the **easiest and most informative** approach to evaluate endothelial function.

✔ **Classical markers** have **limited diagnostic value** when used alone.

✔ **Novel biomarkers** (EMPs, soluble endoglin, endocan) have been studied in various **vasculopathic disorders**.

✔ Among them, **endocan** shows the **highest potential for clinical application**.

✔ A **multibiomarker approach**, combining classical and novel markers, may further improve **risk stratification and patient management** in the future.

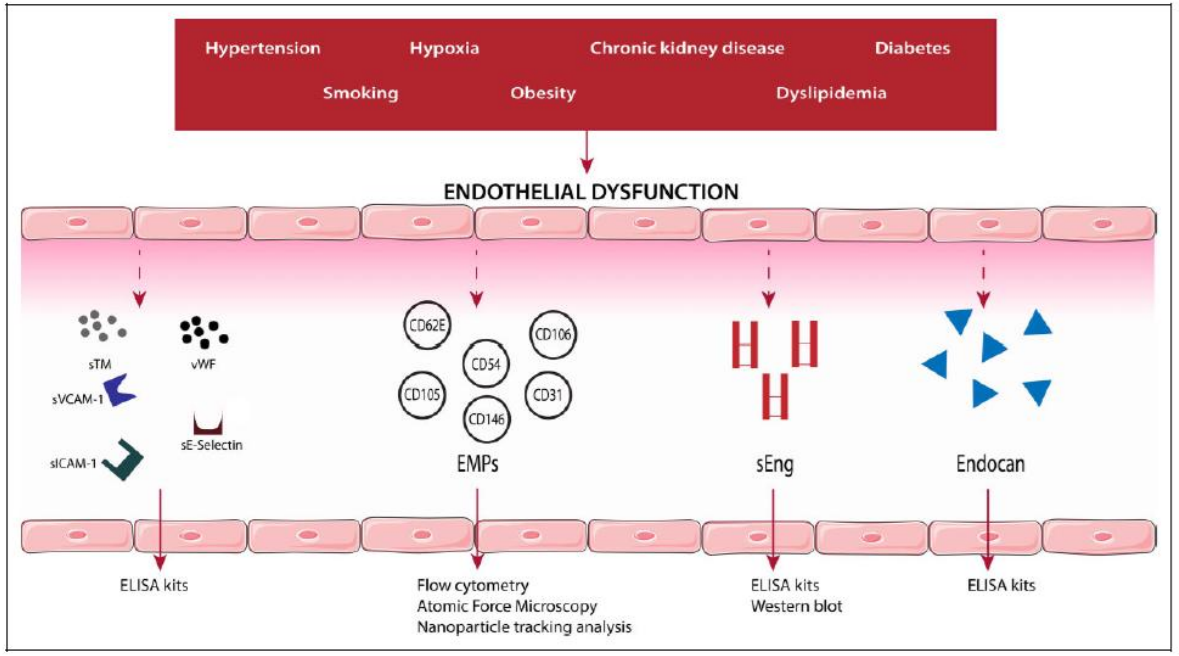


Table 3. Comparison Among EMPs, Endocan, and Endoglin Based on Criteria for an Ideal Endothelial Biomarker.

Criterion	EMP	Endocan	Soluble Endoglin
Reflects endothelial function	Yes	Yes	Probably
Reflects disease state	Yes	Yes	Controversial
Improves CV risk stratification	Yes	Yes	Yes
Specificity	High	High	Moderate
Reproducibility	Weak	High	Moderate
Operator independent	No	Yes	Yes
Inexpensive	No	Yes	Yes
Consensus on definition	No	Yes	Yes
Standardized methodology	No	Yes	Yes

Abbreviations: CV, cardiovascular; EMP, endothelial microparticle.

Source: Leite AR. Angiology. 2020 May;71(5):397-410. doi: 10.1177/0003319720903586.

Endothelial health — the key to vascular balance

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NPOO Institutional research projects Josip Juraj Strossmayer University of Osijek

Endothelial extracellular vesicles as biomarkers of endothelial activation induced by changes in arterial pressure (HYPER-endoEV)

2025-2028

Thank you for your attention