The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine

eJIFCC

12. RATIONAL DIAGNOSIS OF CARDIOVASCULAR DISEASE

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12.1 Introduction

Cardiovascular diseases (CVD) or diseases of the circulatory system represent various clinical conditions due to atherosclerotic impairment of coronary, cerebral or peripheral arteries. The American Heart Association uses the ICD 10 codes I00-I99 including diseases of veins and lymphatics. CVD are considered nowadays as the major causes of death in developed countries for men and women. Detailed epidemiological data for CVD are available from the American Heart Association's "2002 Heart and Statistical Update" summarizing the risk factors. 61,800,000 Americans suffer from one or more types of CVD (29,700,000 males and 32,100,000 females, 24,750,000 older than age 65, details in Table 1) [21].

Cardiovascular disease	Number of patients
All	61,800,000
High blood pressure	50,000,000
Coronary heart disease	12,600,000
Myocardial infarction	7,500,000
Angina pectoris	6,400,000
Stroke	4,600,000
Congenital heart failure	1,000,000
Congestive heart failure	4,790,000

Table 1. Prevalence for cardiovascular diseases in the USA.
Reference: 2002 Heart and Stroke Statistical Update, American Heart Association http://www.americanheart.org/presenter.jhtml?identifier=1928

From these data it was calculated that 1 in 5 citizens have some form of CVD. Comparison of the death rates of various countries (Table 2) shows that there is a relationship between socio-economic situations and a kind of east-west and north-south decline [22]

For the follow-up, diagnosis and risk stratification of patients the diagnostic laboratory in combination with radiology techniques plays a major role. The aim of this review is to describe the diagnostic usefulness of established and new analytes related to cardiac function, coagulation, endothelial dysfunction and risk factors. Some of the strategies mentioned are based on experimental biochemical findings.

	Male Subjects			Female Subjects		
Country	**CVD**	**CHD**	**Stroke**	**CVD**	**CHD**	**Stroke**
Australia	253	172	38	113	61	27
Austria	369	205	59	168	70	38
Canada	380	284	36	122	65	25
France	213	85	41	78	21	22
Germany	347	190	53	149	64	32
Hungary	842	420	207	380	161	111
Italy	293	140	58	116	37	33
Japan	186	57	79	85	20	41
Russia	1167	639	361	540	230	229
Spain	252	121	51	99	32	28
United States	360	230	41	183	95	33

Table 2. Death rates for cardiovascular diseases. Rates per 100.000 population
CVD=Cardiovascular Disease; CHD = Coronary Heart Disease.

Reference: 2002 Heart and Stroke Statistical Update, American Heart Association, http://www.americanheart.org/presenter.jhtml?identifier=1928

12.2 Markers for cardiac function

12.2.1 Acute myocardial infarction

Since 1990 several new markers have been introduced in the diagnostic laboratory for acute myocardial infarction (MI). These analytes are specific cardiac proteins exhibiting a better diagnostic validity than activity of total CK l and CK-MB previously used. There is now consensus among IFCC experts that the most rational way to diagnose MI is the use of a so-called "early" and a "late" marker released from cardiac myocytes under ischaemic conditions. Myoglobin is considered the optimal "early" marker starting to rise 2 to 3 hours and peaking 8 to 12 hours after onset of the clinical symptoms. Normal concentrations are reached again after 24 hours. The cardiac troponins peak at 12 to 48 hours, and remain elevated for 4 to 10 days. According IFCC guidelines, specimen

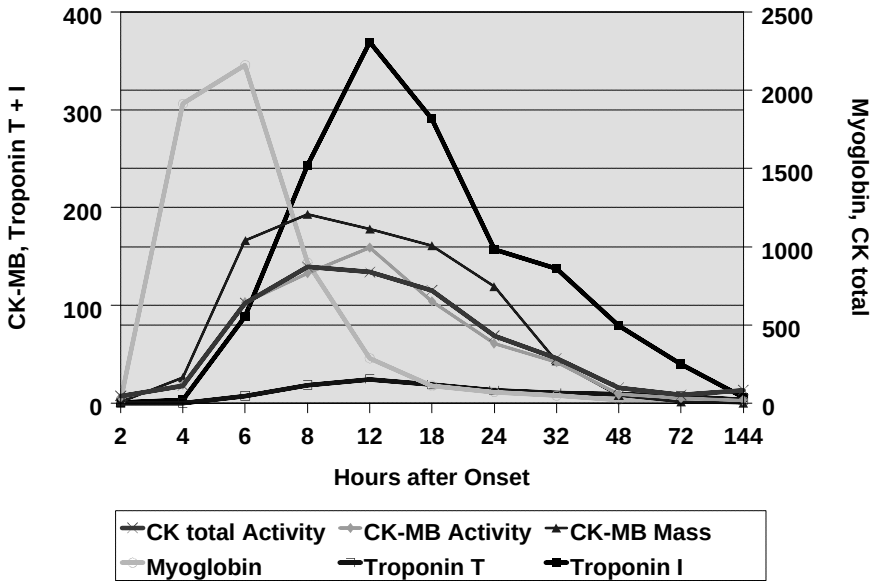


Figure 1. Time course of markers for myocardial infarction

Marker	2h	4h	6h	10 h	14h	18h	22h
Myoglobin							
Sensitivity (%)	26.30	42.90	78.70	86.50	62.30	57.50	42.90
Specificity (%)	87.30	89.40	89.40	90.20	88.30	88.80	91.30
Youden-Index	0.14	0.32	0.68	0.77	0.51	0.46	0.34
Troponin T							
Sensitivity (%)	10.50	35.70	61.70	86.50	84.90	78.70	85.70
Specificity (%)	98.40	98.30	96.10	96.40	96.10	95.70	94.60
Youden-Index	0.09	0.34	0.58	0.83	0.81	0.74	0.80
Troponin I							
Sensitivity (%)	15.80	35.70	57.50	92.30	90.60	95.70	89.80
Specificity (%)	96.80	94.20	94.30	94.60	92.20	93.40	94.20
Youden-Index	0.13	0.30	0.52	0.87	0.83	0.89	0.84
Total CK-MB activity							
Sensitivity (%)	21.10	40.70	74.50	96.20	98.10	97.90	89.80
Specificity (%)	100.00	98.80	97.50	97.50	96.10	96.90	96.20
Youden-Index	0.21	0.40	0.72	0.94	0.94	0.95	0.86
Total CK-MB mass							
Sensitivity (%)	15.80	39.30	66.00	90.40	90.50	95.70	95.70
Specificity (%)	99.20	98.80	100.00	99.60	98.90	99.60	99.10
Youden-Index	0.15	0.38	0.66	0.90	0.89	0.95	0.95

Table 3. Diagnostic validities of markers for myocardial infarction based on time from onset of chest pain Reference: Zimmerman J. et al. [2]

collection should be done at admission, 4, 8, and 12 h (or next morning). In Figure 1 [1] a typical time-course in a patient with MI is shown. According to the diagnostic validities published for CK total, CK-MB, the troponins and myoglobin (Table 3) [2] it is anticipated that after the present transition period clinicians will accept in the future myoglobin and troponin measurements as the gold standards for the diagnosis of MI.

With regard to unstable angina the IFCC and the National Academy of Clinical Chemistry (NACB) have stated that patients with small increases of troponin (the 97.5th percentile of the normal healthy population) should be used as the cut-off point for risk-stratification and further angiographic examinations.

12.2.2 Ventricular dysfunction

For more than 10 years a lot of research focused on the function of the natriuretic peptides which affect systemic blood pressure by several mechanisms, including modification of renal function and vascular tone, counteracting of the renin-angiotensin-aldosterone system and action on brain regulatory sites. These systems maintain a balance that ensures relative constancy of body electrolyte and water content and circulatory homeostasis. The natriuretic peptides (BNP and ANP) investigated since more than 10 years are potential diagnostic tools in the assessment of patients' ventricular function.

Brain natriuretic peptide (BNP), also known as ventricular natriuretic peptide, is a cardiac hormone secreted predominantly from the ventricle. Its biologically active, low molecular form with 32 amino acids (BNP-32, proBNP (77-108), half-life time: 20 min) with vasodilator and natriuretic properties, is cleaved from the proBNP, stored in human cardiac tissue and released from the cardiac ventricles in response to stretching of the chamber.

Group	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
General population aged over 45 (n=307)	100 (65 – 100)	70 (65 – 75)	7 (3 – 14)	100 (99 – 100)
Patients with existing diagnosis of heart failure (n=103)	100 (92 - 100)	18 (10 – 29)	39 (28 – 49)	100 (78 – 100)
Patients at high risk for heart failure (n=133)	100 (72 – 100)	44 (35 – 54)	12 (5 – 21)	100 (96 – 100)

Table 4. Diagnostic validities of NT-proBNP in the general populations and in patients with or at risk for heart failures. Values in parentheses are 95% confidence intervals Reference: Hobbs F. D. et al [5]

The second remnant after cleavage, N-terminal proBNP (NT-proBNP, proBNP (1-76), half-life time: 60 – 120 min), is a 76 amino acid peptide with no known biological function. Plasma levels of NT-proBNP and BNP-32 are similar in normal subjects. In NYHA Class I, II and III subjects, the levels of NT-proBNP are 4-fold higher than concomitant BNP-32 levels. It was shown, that left ventricular ejection fraction (LVEF), exercise-test time and creatinine clearance were independent predictors of NT-proBNP plasma concentrations. The levels of NT-proBNP and BNP-32 were highly correlated. Nevertheless, since the increment above normal levels of NT-proBNP exceeds that for BNP-32 in cardiac impairment it is now agreed that NT-proBNP is a more useful marker of early cardiac dysfunction than BNP-32 and may represent cardiac status over longer periods [3].

NT-proBNP seems to be helpful in the diagnosis of heart failure in the overall population. NT-proBNP is reported to be a very useful tool [4] especially for the detection of left ventricular systolic dysfunction. The diagnostic validities for NT-proBNP in the general population, in patients with existing heart failures and with risks for heart failure are shown in Table 4 [5]. Because of its high negative predictive values patients with a positive result should be sent to the clinicians for cardiac imaging. Distinguishing dyspnoea due to congestive heart failure is also discussed as a potential application of BNP measurements [6].

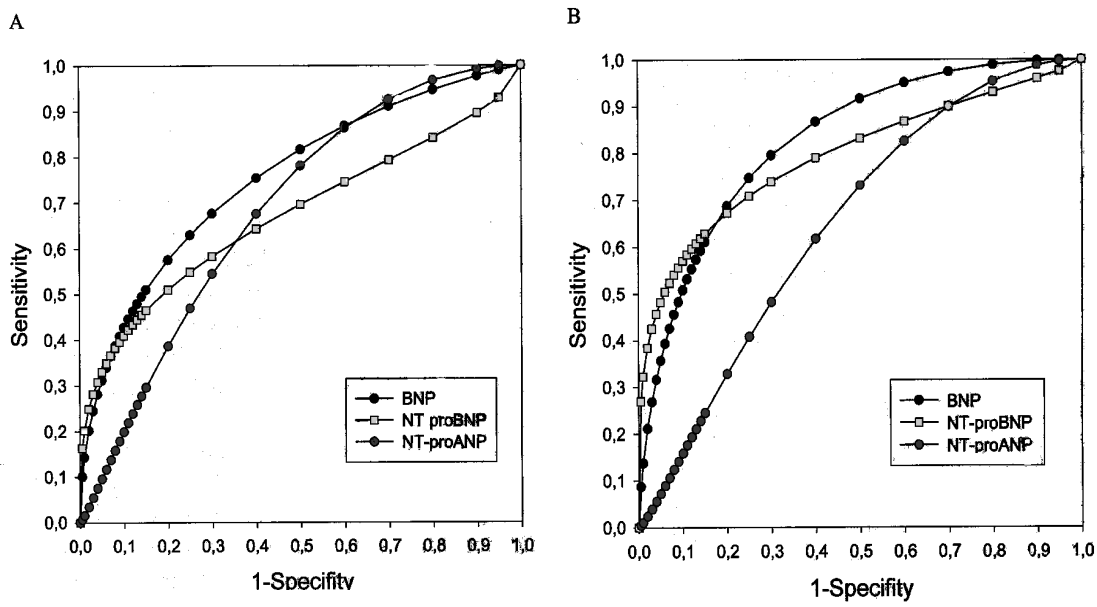
The atrial natriuretic peptides (ANP) exhibit similar physiological functions than BNP. ProANP (1-126) is stored in membrane-bound granules in artrial cardiocytes. Upon stimulation, these granules move to the cell surface, releasing the stored proANP (half-life time 60 min). This prohormone is cleaved into the active 28 amino acid peptide ANP (pro ANP (99-126), a-ANP, half-life time: 2.5 min), and an N-terminal ANP fragment (NT-proANP, proANP (1-98) which is further processed within the circulation to form proANP (1-30), proANP (31-67) and proANP (79-98). Due to much longer biological half proANP moieties exhibit up to 50 times higher plasma concentration than a-ANP.

Several studies reported that plasma-levels of a-ANP and NT-proANP were significantly elevated even in asymptomatic patients with left ventricular dysfunction. A comparison of the new cardiac markers NT-proANP, BNP and NT-proBNP demonstrate the efficiency for diagnosis of patients with impaired left ventricular ejection fraction (LVEF) (Table 5, Fig. 2) [7, 8].

Statistics	BNP Cut-off: 41 pmol/l	NT-proBNP Cut-off: 488 pmol/l	NT-proANP Cut-off: 2150 pmol/l
Sensitivity (%)	73	70	59
Specificity (%)	77	73	61
Pos. Predictive Value (%)	70	61	48
Neg. Predictive Value (%)	79	80	71
Efficiency (%)	75	72	60

Table 5. Diagnostic performance of BNP, NT-proBNP and NT-proANP at optimal cut-off for discriminating two groups of patients with left ventricular ejection fraction (LVEF) values < 40% versus > 40% Reference: Maeda K. et al. [7]

This clinical study shows on basis of the ROC curves that NT-proANP might be the most useful non-invasive marker. Based on the clinical investigations, measurement of natriuretic peptides is useful for those clinical conditions summarized in Table 6. Even in clinically asymptomatic patients the peptides were reported to be of value.



A: LVEF < 48 % based on 3D-echo-cardiography and < 55 % based on RNV

B: Resting LVEF < 40 % based on RNV.

Figure 2. ROC curves for BNP, NT-proBNP and NT-proANT in patients with impaired left ventricular ejection fraction. Reference: Hammerer-Lercher A. et al. [8]

Cardiac disease / condition	NT-proANP	NT-proBNP
Assessment of cardiac condition (symptomatic, asymptomatic)	++	++
Screening for NYHAN I patients	+	+
Diagnosis of left ventricular dysfunction	+	+
Diagnosis of heart failure	+	+
Diagnosis of ventricular hypertrophy		+
Prognosis of myocardial infarction	+	+
Diagnosis of pre-eclampsia	+	+
Diagnosis of essential hypertension	+	+
Monitoring of ACE-inhibitors therapy		

Table 6. Natriuretic peptides in heart function assessment

12.3 Markers for thrombophilia

Thrombophilia can be defined as an increased tendency for venous and arterial thrombo-embolic events. Thrombophilia occurs from very different biochemical, genetic and immunological abnormalities and is induced by mechanical or rheological adverse events. Thrombo-embolic episodes due to plasmatic hypercoagulation and hypofibrinolysis are often associated with surgery, immobilization, autoimmune disease, impaired glucose tolerance or type 2 diabetes mellitus resulting in reduced endothelial thrombotic resistance and hyper-reactivity of thrombocytes. The various conditions associated with thromboembolic events are summarized in Table 7. Defining the cause of hypercoagulability may determine the type and duration of treatment for the associated thromboembolic events. In addition, finding a genetic defect in coagulation allows for testing at least the first-degree asymptomatic family members.

Congenital deficiencies / mutations	Acquired factors / secondary
Antithrombin III	Anti-phospholipid antibodies (Autoimmune)
APC Resistance (Factor V Leiden)	APC Resistance
Hyperhomocysteinemia Cystationine-ß-synthetase Methionine synthetase Methylene-tetrahydrofolate reductase	Hyperhomocysteinemia (vitamin deficiency)
Protein C	Pregnancy or other conditions Deficiencies: Protein C, S
Protein S	Increased coagulation factors: Fibrinogen Factors VIII, IX, XI
Prothrombin G 20210A mutation	

Table 7. Congenital and acquired factors associated with thromboembolic events

12.3.1 Rational screening

Several clinical investigations have been conducted to determine the prevalence of the various coagulation factor defects in the general population. All these changes in the plasmatic coagulation lead to a several fold increase in the risk for developing thromboembolic events. In Table 8 [9, 10, 11] the clinical knowledge gathered so far is summarized. It is obvious that the risk for thrombosis in families showing one or even more defects is usually 4- to 8-fold higher compared to the general population.

Based on these facts and figures the diagnostic laboratory is requested to perform a kind of rationale step-by-step diagnosis in patients with clinical symptoms and or a family history of thromboembolic diseases (deep venous thrombosis, stroke, pulmonary embolism). Investigation of a patient always starts with the global (routine) coagulation tests, followed by screening for the protein C pathway (Figure 3) and homocysteine plasma levels, finally ending-up with the determination of the single coagulation factors and antiphospholipid antibodies. Usually a prolonged activated prothrombin time due to consumption of coagulation factors or deficiencies is one of the first hints of a disturbance. It is stressed that hypercoagulability disorders can be diagnosed in approximately 80 % of patients suffering from thrombotic events.

12.4 Markers of endothelial function

Over the last twenty years, researchers have found an extraordinary variety of endothelial functions, including control over coagulation, fibrinolysis, vascular tone and growth as well as immune responses [12]. With regard to its central location, its integrating and transducing capability, and the large repertoire of its biologically active products, the endothelium plays a main role in a series of pathophysiological balances (Figure 4). It is well accepted that the repeated injury or lack of the endothelium is responsible for local activation of platelets, activation of coagulation and release of growth factors which lead to intimal hyperplasia, lipid accumulation and formation of atherosclerotic lesions. The well-known consequences of theses lesions are the development of cardiovascular diseases.

12.4.1 Endothelium and coagulation

In an antagonistic manner dependent on the environment the endothelial monolayer influences platelet function, plasmatic coagulation and fibrinolysis by a variety of anti- and procoagulant products and metabolites released being normally in a balance (table 9). A crucial physiological function of the endothelium is to facilitate blood flow by providing an antithrombotic surface that inhibits platelet

Table 8. Incidence of congenital defects and increased coagulation factors in the general population and the increased relative risks for thromboembolism

Deficiencies /mutations	Incidence in the population %	Relative risk of thromboembolism
Antithrombin III	0.02 – 0.17	8
APC Resistance Factor V Leiden	Heterozygote: > 20 Homozygote: 3.6 – 6.0	2 – 8 100
Hyperhomocysteinemia (MTHF reductase)	Heterozygote: 5- 10 Homozygous: 40	
Protein C	0.14 – 0.5	8
Protein S	< 1.0	8
Prothrombin 20210A	1- 3	2 – 4
Conditions		
Factor VIIIc > 1500 U/L	11	2 - 4
Factor IX > 1280 U/L		2.5
Factor XI > 1200 U/L		2.2
Fibrinogen > 5 g/L		4.0

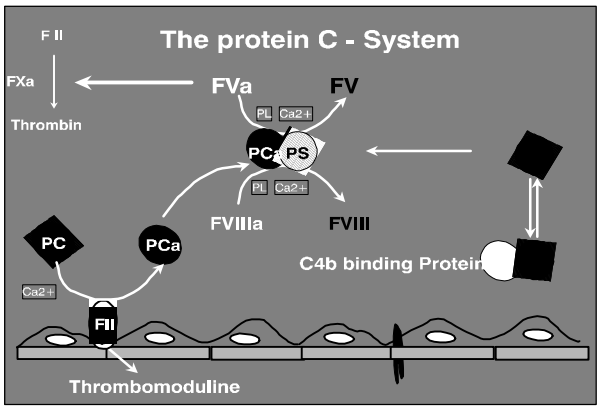


Figure 3. The protein C pathway

adhesion and clotting [12]. This is due to the negative charged layer of proteoglycans, by inhibition of platelet aggregation by prostacyclin (PGI_2) and endothelium derived relaxing factor (EDRF) and by inhibition of plasmatic coagulation. However, when the endothelium is perturbed by physiological forces or by specific chemical factors, the cells undergo programmatic biochemical changes that culminate in their transformation to a prothrombotic surface (Table 9).

Factor VIII-vWF as well as thrombomodulin can serve as a marker of endothelial dysfunction in several diseases demonstrating probably imbalance. For example in patients suffering from peripheral vascular disease significantly elevated plasma levels in comparison to healthy controls are reported (Table 10) [13]. In patients with a peripheral arterial occlusive disease thrombomodulin measured at entry is reported to be significantly higher in those patients developing late re-stenosis [14]. From these results increased plasma thrombomodulin levels seem to be a hint for hypercoagulability.

12.4.2 Endothelium and vascular tone

Endothelial cells secrete mediators that influence the vascular tonus and hemodynamics (Table 11) [12]. The switch from a normally predominant release of relaxing factors to that of contracting factors is suggested to play a key role in atherosclerosis. Release of NO from endothelial cells is enhanced by physical and chemical stimuli such as pulsatile blood flow, hypoxia, free radicals, acetylcholine, thrombin, serotonin, histamine, substance P and bradykinin [12]. NO acts as a kind of anti-vasoconstrictor to stimuli such as free radicals during oxidative stress and inflammation responsible for release of endothelin I and ACE, the most potent to vasoconstrictors. The latter are increased under various clinical conditions and most probably triggered by immunological and inflammatory stimuli; increased blood levels of ET-1 are seen in patients with congestive heart failure (CHF) and after heart transplantation [15]

The systemic hypotension observed in septic patients is related to an increase in NO production. The imbalance of the vascular tonus by excessive endothelin I and NO productions due to endothelial dysfunction is clearly demonstrated in patients suffering from bacterial septic infections (Figure 5) [16]. NO measured as NO^{2-}/NO^{3-} is a good predictor of all shock conditions [17].

12.4.3 Endothelium and immune response

Vascular endothelial cells play an important part in combination with blood cells during the activation of the immune system. There exists a complex system: cytokines excreted from lymphocytes and monocytes stimulate

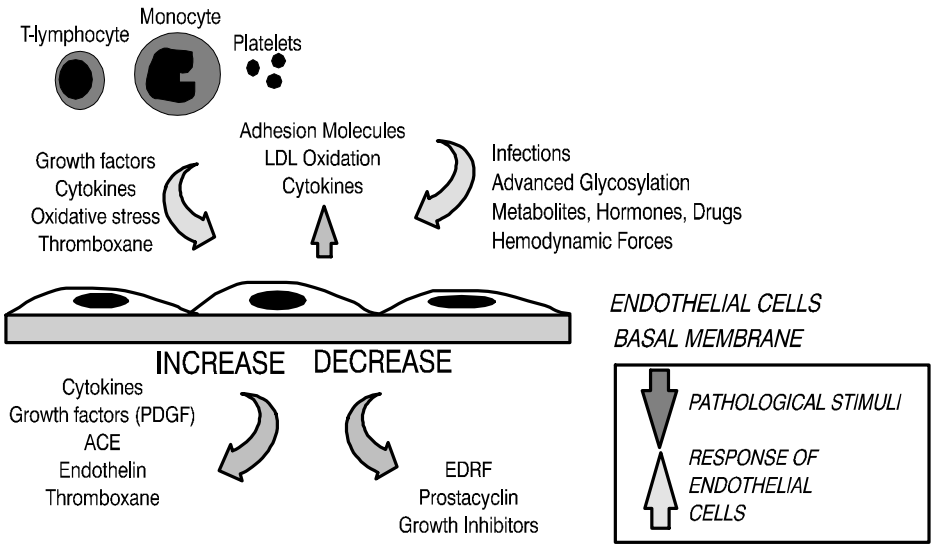


Figure 4. Pathological mechanism for endothelial activation and dysfunction

endothelial cells to release cytokines too, eicosanoids, and adhesion molecules. The up-regulation and expression of adhesion molecules result in chemo attraction of mononuclear cells and facilitate their penetration through the endothelial monolayer to the intimae, a process important for parthenogenesis (12). Marked increases in circulating levels of adhesion molecules were observed in hyperlipidaemia, hypertension, diabetes mellitus, under oxidative stress, and cigarette smoking (12,18,19). Increased plasma levels of the adhesion molecule P-selectin in patients with hypertension demonstrate the progressive vascular damage due to increased sheer stress (Figure 6) (19) and most probably concomitant with adhesion of leukocytes on the endothelial surface.

Inhibitor of coagulation	Promoters of coagulation
Prostacycline	Thromboxane A_2
Thrombomodulin	Platelet activating factor
Heparin-Proteoglycans	Tissue factor
Tissue plasminogen activator	Plasminogen activator inhibitor
Urokinase	Factor VIII von Willebrand

Table 9. Endothelial cells and coagulation

The physiological function of the endothelium is rather complex and the various functions described occur simultaneously in order to keep a homeostatic condition. Several molecules mentioned as markers of acute and chronic endothelial activation with subsequent dysfunction of the endothelium are considered to play a role in atherosclerosis. They may furthermore serve as a tool to monitor the impact of prevention and intervention on vascular damage.

Marker	Controls	Patients
Factor VIII von Willebrand (U/L)	1020 ± 300	1330 ± 340 *
Thrombomodulin (ng/ml)	46 ± 14	55 ± 15 **

Table 10. Markers of endothelial dysfunction in patients suffering peripheral vascular disease

12.5 Risk factors

Oxidative stress as a result of the enhanced formation of reactive oxygen species (ROS) due to infections or immune response plays a central role in the pathogenesis of arteriosclerosis associated diseases. Hypercholesterolaemia, hyperlipidaemia, hypertension, cigarette smoking, diabetes mellitus, and the chronic activation of the immune system are classical conditions where enhanced ROS concentrations are observed. The measurements of thiobarbituric acid reactive substances (TBARS) are serving since several years as measure for these pathological conditions, since oxidative species are essential for the

Relaxors	Constrictors
Prostacyclin	Endothelin 1
Nitric oxide (EDRF)	Angiotensin II (formed by ACE)
EDRF like substances	Platelet derived growth factor

Table 11. Endothelial cells and vascular tonus

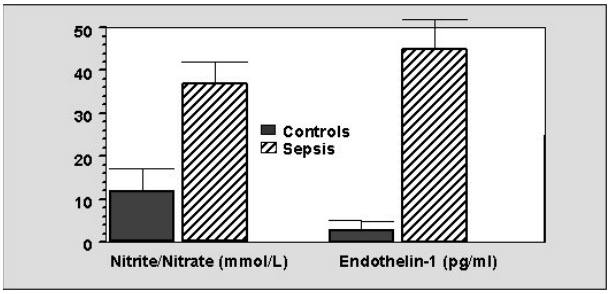


Figure 5. Disturbance of endothelial tonus signaling in septic patients: NO and endothelin-1 plasma levels. Reference: Avontuur JA et al [16]

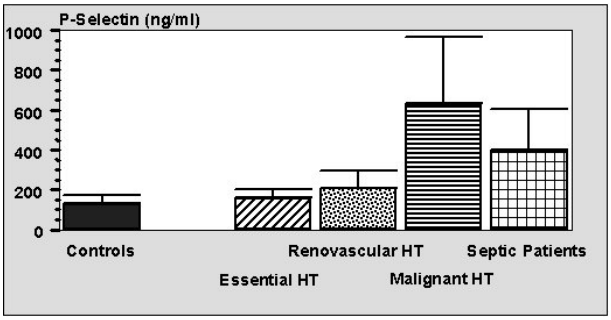


Figure 6. Plasma levels of the adhesion molecule P-selectin in patients with hypertension. Reference: Verhaar MC et al [19]

modification of lipoproteins and their atherogenic effects. In diabetics with peripheral vascular diseases and/or coronary artery diseases plasma TBARS were significantly elevated (Table 12) (20). Their increase was more pronounced than the lipids investigated and correlated with the patients' clinical conditions.

12.6 Conclusion

It was the aim of this review to highlight established markers of cardiovascular diseases used for the diagnosis of myocardial infarction and for the diagnosis of thromboembolic events. In addition some new molecules were described as potential new markers of dysfunction of the endothelium and thus being relevant for atherogenesis.

Parameter	Healthy persons (n=62)	Type I controls (n=62)	Type I with PVD or CAD (n=15)	Type II controls (n=42)	Type II with PVD or CAD (n=39)
HbA_{1c} (%)	3.8 (2.8 – 5.5)	9.4*# (8.0 – 10.2)	8.0*# (6.9 – 8.8)	9.2* (7.6 – 10.5)	8.9* (7.7 – 9.6)
Total cholesterol (mmol/L)	5.7 (5.5 – 6.3)	5.0 (4.1 – 6.0)	6.2 (4.9 – 6.7)	5.4+ (4.4 – 6.3)	6.4+ (5.4 – 7.3)
Triglycerides (mmol/L)	1.2 (1.1 – 1.7)	1.1 (0.9 – 1.6)	1.2 (1.0 – 1.6)	1.2+ (1.0 – 1.9)	2.1*+ (1.5 – 3.1)
TBARS (µmol/L)	5.4 (4.0 – 6.9)	9.1* 7.5 . 11.9	9.2* (6.7 – 10.6)	10.1*+ (7.9 – 13.0)	13.9*+ (11.4 – 17.2)
∑TBARS/(CHOL+TG)	0.7 (0.6 – 0.9)	1.4* (1.2 – 1.8)	1.3* (1.1 – 1.5)	1.4* (1.1 – 1.9)	1.6* (1.2-1.9)

Table 12. Biochemical data for diabetics with and without peripheral vascular diseases (PVD) or coronary artery diseases (CAD)
medians (interquartile ranges Q1 – Q3)

*p < 0.005 compared to healthy individuals

#p < 0.005 between type I patients with and without PVD/CAD

+p < 0.005 between type I patients with and without PVD/CAD

Reference: Griesmacher A et al [20]

For many of them, however, the mechanisms underlying the genesis of circulating forms, as well as their pathophysiological significance, remain unclear so far. Further studies are necessary to establish the diagnostic relevance of these serum markers of endothelial function in large scale clinical investigations. They may be additional tools in the risk stratification of cardiovascular diseases.

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