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Acute heart failure syndromes

-treatment, outcomes and pathophysiological aspects of inflammation and vascular function

Stig Eggen Hermansen

A dissertation for the degree of Philosophiae Doctor
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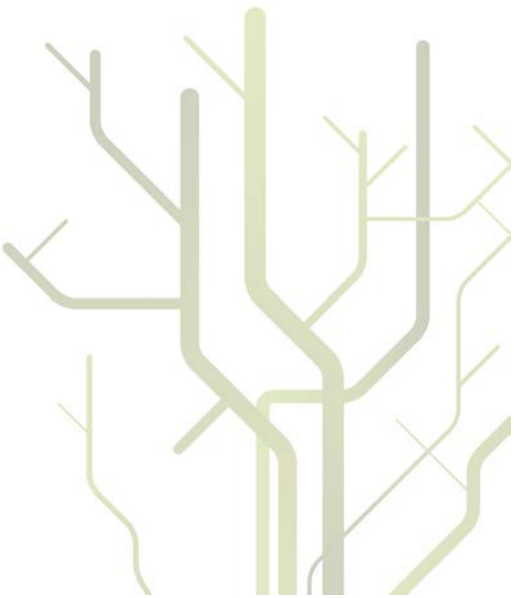

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Contents

1. Acknowledgements

The experimental and laboratory parts of this work were carried out between 2004 and 2010 at the Department of Clinical Medicine and Department of Medical Biology at the Faculty of Medicine, University of Tromsø. The retrospective registries and human observational studies were carried out at the Department of Cardiothoracic and Vascular Surgery (Division of Cardiothoracic and Respiratory Medicine), Department of Anesthesiology (Division of Surgical Medicine and Intensive Care) and Department of Cardiology (Division of Cardiothoracic and Respiratory Medicine) at the University Hospital of North Norway. As the majority of my predecessors I was privileged to combine research and scientific work with clinical work and education at the Department of Cardiothoracic and Vascular surgery. This work was supported by Helse Nord and the Northern Norway Regional Health Authority.

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2. List of papers

I. Hermansen SE, Hansen M, Roaldsen M, Muller S, How OJ, Myrmel T. **Utilization and outcome of coronary revascularization and valve procedures in acute heart failure--an evaluation based on the classification from the European Society of Cardiology.** Interact Cardiovasc Thorac Surg 2008;7:833-8.[1]

II Hermansen SE, Hansen M, Roaldsen M, Muller S, How OJ, Myrmel T. **How many acute heart failure patients need a ventricular assist device?** Scand Cardiovasc J 2008;42:118-24. [2]

III Hermansen SE, Hansen M, Muller S, How OJ, Myrmel T. **Endothelial function during open heart surgery** (manuscript)

IV Hermansen SE, Kalstad T, How OJ, Myrmel T**. Inflammation and reduced endothelial function in the course of severe acute heart failure.** Transl Res 2011;157:117-27.[3]

V Hermansen SE, Lund T, Kalstad T, Ytrehus K, Myrmel T. **Adrenomedullin augments the angiogenic potential of late outgrowth endothelial progenitor cells.** Am J Physiol Cell Physiol 2011 Jan 5.[4]

Appendix

Hermansen SE, Naesheim T, How OJ, Myrmel T. **Circulatory assistance in acute heart failure--where do we go from here?** Scand Cardiovasc J 2009;43:211-6.[5]

3. Selected abbreviations

ADHERE	Acute Decompensated Heart Failure National Registry
ADMA	Asymmetrical dimethylarginine
AHF	Acute heart failure
ALARM-HF	Acute Heart Failure Global Registry of Standard Treatment
AM	Adrenomedullin
AMIS Plus Registry	Acute Myocardial Infarction in Switzerland
CABG	Coronary artery bypass grafting
CCU	Coronary care unit
CS	Cardiogenic shock
ECMO	Extracorporeal membrane oxygenation
ECS	European Society of Cardiology
EFICA	Etude Francaise l'Innsuficiens Cardiaque Aigue
EHFS	EuroHeart Failure Survey
EPC	Endothelial progenitor cells
GRACE	Global Registry of Acute Coronary Events
IABP	Intra-aortic balloon counterpulsation
ICU	Intensive care unit
LVAD, VAD	Left ventricular assist device, ventricular assist device
MR	Mitral regurgitation
NRMI	National Registry of Myocardial Infarction
PC-HF	Postcardiotomy heart failure
PCI	Percutaneous coronary intervention
RH-index	Reactive hyperemia index
VEGF	Vascular endothelial growth factor

4. Introduction

During the last three decades there has been extraordinary progress in the understanding of the pathogenesis, and most importantly, the treatment of ischemic heart disease. This has resulted in impressive improvements in survival for patients hospitalised with acute myocardial infarction. However, in this era of myocardial reperfusion, *cardiogenic shock* is still the leading cause of death for patients hospitalised with acute myocardial infarction. Mortality rates for patients in cardiogenic shock are still exceedingly high, demonstrating the limitations of early and aggressive revascularization. Moreover, there is a growing epidemic of patients with chronic heart failure and increasing numbers of patients with acute-on-chronic heart failure necessitating hospitalisation. Of concern amidst the general improvements, however, the most recent clinical trials have all failed to improve outcomes for patients with acute heart failure, and new insights into the underlying pathophysiology and therapy for these syndromes are needed. The present work was initiated to describe the clinical epidemiology of acute heart failure; i.e. incidence, treatments employed and outcome. Also, to improve the pathophysiological understanding of the acute heart failure syndromes, we have done a number of exploratory assessments of the integrated inflammatory and vascular responses in patients with the most severe form of acute heart failure, namely cardiogenic shock and postcardiotomy heart failure.

5. Background

5.1 Acute heart failure

The European Society of Cardiology (ESC) published in 2005 the first set of guidelines on the diagnosis and treatment of acute heart failure.[6] The definition of acute heart failure used in these guidelines was:

"The rapid onset of symptoms and signs secondary to abnormal cardiac function. The cardiac dysfunction can be related to systolic or diastolic dysfunction, to abnormalities in cardiac rhythm, or to pre-load and after-load mismatch. It is often life threatening and requires urgent treatment"

ESC further divided AHF into a set of six distinct clinical conditions: 1) Acute decompensated heart failure, 2) Hypertensive acute heart failure, 3) Pulmonary edema, 4) Cardiogenic shock, 5) High output failure, 6) Right heart failure.

The broad spectrum and heterogeneity of different AHF conditions are apparent from this classification. There is a great span from patients with acute decompensated heart failure, which by definition present with mild AHF symptoms, to cardiogenic shock with regards to clinical presentation, prognosis and treatment.

5.2 Epidemiology of acute heart failure

There have been several publications aiming to describe the AHF population, including the major EuroHeart Failure Survey II (EHFS II) and the US Acute Decompensated Heart Failure National Registry (ADHERE).[7-12] The EuroHeart Failure Survey II included 3580 patients with AHF hospitalized in 133 European hospitals from 2004-2005. The aim was to give a description based on the European Society of Cardiology (ESC) guidelines on the diagnosis and treatment of AHF.[12] The overwhelming majority of patients had mild forms of AHF and the main precipitating factors for AHF were acute coronary syndromes (30 %), arrhytmias (32 %) and valvular pathology (27 %). AHF patients were typically elderly (mean age 69.9 years) and more than two-thirds had a prior history of heart failure. Pre-existing medical conditions were abundant, such as coronary heart disease (54 %), hypertension (63 %), atrial fibrillation/flutter (39 %), valvular disease (34 %) and diabetes mellitus (33 %). The overall

in-hospital mortality was 6.7 % in EHFS-II. Other surveys on AHF report varying mortality rates: ADHERE (4.0 %)[10], Italian AHF survey (7.3 %)[9] and ALARM-HF (12 %)[8]. Although the in-hospital mortality in mild forms of AHF is modest, an episode of AHF requiring hospital treatment is associated with a dismal intermediate and long term prognosis and as such identifies patients at increased risk.[13]

The in-hospital prognosis of patients requiring treatment at the ICU and CCU is, however, worse. The French EFICA study "Etude Francaise l'Innsuficiens Cardiaque Aigue" found in this more acutely ill AHF population an overall four week mortality of 27 %. The international Acute Heart Failure Global Registry of Standard Treatment (ALARM-HF) confirmed this finding with an in-hospital mortality rate of 18 % and 11 % for ICU and CCU treated patients, respectively.[8]

Epidemiology of cardiogenic shock

The most severe form of AHF, cardiogenic shock, stands out with excessively high short term mortality. The hospital mortality rates for these patients, regardless of cause, have been reported in the most recent surveys to lie just below 50 %[12, 14-16] and the vast majority of deaths are cardiac[17]. ACS was the predominant etiology (72 %), while valvular dysfunction was a precipitating factor in 17 % in EHFS-II. Compared to the general AHF population, CS patients were slightly younger (mean age 67 years) and predominantly males (68 %). The majority had no prior history of heart failure (65 %), but the frequency of underlying medical conditions was largely similar to other AHF conditions. The overall incidence of CS in this survey was 4 %. The incidence of CS following ACS in larger registries is reported to lie between 8-9 % for STEMI[14, 16, 18] and 2-3 % for non-STEMI ACS[14, 19, 20]. Although reports are conflicting, the incidence and in-hospital mortality of CS following AMI seems to be

declining.[14, 19, 21] Importantly, long term follow-up have established that patients who survive hospitalisation have a relatively good long term survival and also quality of life.[22, 23]

Epidemiology of postcardiotomy heart failure

Acute heart failure following open heart surgery is virtually not mentioned in the current AHF guidelines and large registries. However, this entity shares a number of common epidemiological and pathophysiological aspects with acute heart failure of non-surgical etiology. Predictors for developing postcardiotomy HF include low preoperative left ventricular ejection fraction, repeat surgery, emergency operation, female gender and high age.[24] Depending on the definition used, postcardiotomy HF is reported to occur in 2 -9 % of all open heart operations with mortality rates ranging from 17 – 34 %.[24-26] There is, however, a great spectrum of patients within this group ranging from patients presenting with mild AHF syndromes to patients presenting with postcardiotomy shock refractory to conventional treatments. In this latter group, in-hospital mortality rates as high as 80 % was observed in patients requiring multiple high doses of inotropic drugs.[27] In the current registries and patient series the overall percentage of open heart surgical procedures necessitating ventricular assist systems for circulatory support was 0.2-1.2 %.[27-33] The reported outcomes for these patients are highly variable with survival rates to discharge ranging from 25-60 %.[27-33] However, the largest report to date from the US "Society of Thoracic Surgeons` National Cardiac Database" demonstrated significantly improved survival rates for these patients from 1995 through 2004.[30] In this report mean age was 63 years, predominately male patients and most patients did not have prior heart failure before surgery.

5.3 Pathophysiology and definitions of cardiogenic shock and postcardiotomy heart failure

Cardiogenic shock is characterized by a failure of the heart to deliver a sufficient cardiac output to maintain adequate perfusion of vital organs with subsequent inadequate cellular metabolism. Irreversible cell damage and organ failure are the consequences. The basic clinical diagnosis of this condition is typically based on the presentation of arterial hypotension and signs of reduced tissue perfusion. Impaired perfusion of the kidney, brain and skin result in oliguria, altered mental status accompanied by cold and clammy skin. The ESC guidelines on acute heart failure suggested the following definition[6]:

> *"Cardiogenic shock is defined as evidence of tissue hypoperfusion induced by heart failure after correction of pre-load. It is usually characterized by reduced blood pressure (systolic BP < 90 mmHg or a drop of mean arterial pressure > 30 mmHg) and/or low urine output (<0.5 ml/kg/h), with a pulse rate > 60 b.p.m with or without evidence of organ congestion. There is a continuum from low cardiac output syndrome to cardiogenic shock"*

A similar, but more exact definition with regards to hemodynamic variables was used for inclusion of patients in the seminal trial Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock (SHOCK).[34] This definition is often referred to in recent publications concerning patients with cardiogenic shock:

> *"The clinical criteria were hypotension (a systolic blood pressure of < 90 mmHg for at least 30 minutes or the need for supportive measures to maintain a systolic blood pressure of >= 90 mmHg) and end-organ hypoperfusion (cool extremities or a urine*

output of < 30 ml per hour, and a heart rate >= 60 beats per minute). The hemodynamic criteria were a cardiac index of no more than 2.2 L/m2 and a pulmonary-capillary wedge pressure of at least 15 mmHg"

The definition of CS is complicated by the wide array of support measures initiated early to improve hemodynamics and maintaining organ function in these patients. Thus, objective hemodynamic assessment and clinical findings sometimes reflects medically and mechanically supported patients that most likely would succumb without support. This explains some of the variability in the definitions of cardiogenic shock used in registries and clinical trials, but also complicates the diagnosis and treatment of these patients in clinical practice.

Postcardiotomy HF is usually defined as inadequate cardiac performance following heart surgery requiring inotropic and/or intra-aortic balloon counterpulsation support.[24] However, the definitions used vary and the general definitions of AHF syndromes may also be applicable to describe heart failure following surgery. Of importance, right ventricular dysfunction is present in up to 40 % of post surgical patients who develop shock and is associated with high mortality.[35] Of importance, a large proportion of patients with shock following open-heart surgery are in a state of vasodilatory shock with a relatively preserved cardiac function.[36]

While invasive hemodynamic parameters obtained by right heart catheterization (Swan-Ganz catheter) represent a gold standard to ensure a correct diagnosis, evaluation with echocardiography has reduced the need for invasive measures. Invasive and echocardiographic hemodynamic indicators provide important information about cardiac function and central blood flow. Importantly, short term outcome is directly related to the severity of hemodynamic derangement.[37, 38] This is also the case for revascularized patients.[38]

Prognosis is also associated with the initial left ventricular systolic function and severity of mitral regurgitation as assessed by echocardiogrphy[39] Hemodynamic measurements do not, however, provide evidence of end-organ hypoperfusion, the hallmark of shock. Besides the mentioned clinical signs of hypoperfusion, the diagnosis can be supported by increased lactate and lactacidosis as a result of tissue hypoxia and anaerobic metabolism. Hyperlactatemia is a sign of profound and persisting shock and provides prognostic information on the patient.[40] Also, the mixed venous oxygen saturation is typically reduced in low output shock and reflects an imbalance between oxygen delivery and uptake.

In the SHOCK Trial registry including patients with cardiogenic shock after acute myocardial infarction, predominant left ventricular failure was seen in 79 %, isolated right ventricular failure in 3 %, severe mitral regurgitation in 7 %, ventricular septal rupture in 4 % and tamponade in 1.4 %.[41]

The classical cardiogenic shock occurs after a massive and extensive myocardial infarction with a loss of ventricular mass > 40 % leading to severe left ventricular dysfunction, pump failure and subsequently reduced cardiac output and blood pressure.[42-44] The compensatory neurohormonal responses lead to vasoconstriction, tachycardia and fluid retention which in turn can lead to a progressive worsening of coronary blood flow and myocardial function. This classical description of cardiogenic shock does not necessarily correspond with today's patients. Today's patients are often revascularized by means of PCI or CABG, mechanically ventilated, they are old (mean age 67 years EHFS-II) and have multiple comorbideties including pre-existing heart failure. Also, many of the patients receiving full invasive treatment today would not be considered for treatment even a few decades ago. Importantly, observations from the SHOCK trial and registry demonstrated a wide variation in left ventricular ejection fraction (average 30 %) and systemic vascular resistance in patients with shock suggesting that the pathophysiological mechanisms of

cardiogenic shock may vary and not be explained fully by traditional models.[45] Also, the average systemic vascular resistance (SVR) was not elevated and one-fifth of the patients showed clinical signs of severe systemic inflammation with reduced SVR.[46, 47] On the other side of the spectrum, a subset of patients also show signs of hypoperfusion following AMI with elevated SVR and without hypotension.[47]

The notion that a number of patients with CS presented with signs of systemic inflammation and that systemic vascular resistance was inappropriately low leads to a new hypothesis; that the inflammatory response through induction of excessive NO production inhibited compensatory vasoconstriction and impaired myocardial function contributing to persistence of shock.[45]

Treatment refractory cardiogenic shock

Profound shock at presentation or refractory and persistent cardiogenic shock despite patent revascularization, IABP and inotropes/vasopressors, is a subgroup of patients overrepresented among the fatalities. Sheu et. al found CS patients in profund shock despite inotropic and IABP support before PCI to have a substantially higher mortality (71 % vs 22 %).[48] Similar high mortality rates were found in non-responders to vasopressors after PCI (68 % vs 20 %)[49] and patients with the highest vasopressor needs had an excessively high mortality (86 %)[50]. A high need for vasopressors was also predictive of poor outcome in patients from the Triumph trial with persistent CS after successful revascularization.[51]

As discussed above, a significant subset of CS patients does not have an effective vasoconstriction with increased systemic vascular resistance despite vasopressor therapy. A vasodilatory shock with poor response to vasopressors can occur secondary to any type of severe and long lasting shock.[52] This inappropriate vasodilatation is also evident in patients following cardiopulmonary bypass or treatment with ventricular assist devices.[36] There are

several proposed mechanisms behind the vasodilation in vasodilatory shock, including unregulated (increased) NO synthesis, deficiency of vasopressin secretion and lactic acidosis causing vascular smooth muscle dilation.[52]

5.4 Invasive treatments for acute heart failure.

Revascularization

Acute coronary syndromes were a precipitating factor for AHF in one-third of all patients in the EHFS-II registry.[12] This was even more frequent in patients who presented with pulmonary edema (49 %) and cardiogenic shock (72 %). Half of the patients with CS presented with STEMI, while non-STEMI were mostly found in patients with pulmonary edema. Existing guidelines advocate an early invasive revascularization strategy for patients with CS.[6, 53-55] A reported invasive revascularization frequency in EHFS-II around two-thirds of patients with CS secondary to ACS, indicates a restrictive utilization of invasive revascularization compared to existing guidelines.[12] Of even more concern, in the "Global Registry of Acute Coronary Events" (GRACE) and "Euro Heart Survey of Acute Coronary Events" (EHS ACS 1), a 40 - 43 % revascularization rate for AMI complicated with CS was observed.[56, 57]

Previous registry reports have demonstrated that early invasive revascularization is beneficial even in clinically selected elderly patients, but this has not been confirmed in randomized trials.[16, 56, 58] The ACC/AHA guidelines give a class IIa recommendation for early revascularization in selected elderly patients with STEMI [59], but clear evidence guiding the choice of revascularization strategy in non-STEMI presenting with CS, is lacking [60].

The role of CABG in the acute phase of STEMI and CS is somewhat unclear, but the AHA/ACC guidelines state that CABG should be used if there is a suitable anatomy (main

stem, three vessel disease) and if PCI fails to adequately reperfuse the myocardium or cannot be done.[59, 61] Importantly, approximately 40 % of the patients revascularized early in the SHOCK trial were treated with CABG with no higher mortality than patients treated by PCI. [62] There are, however, no robust data comparing PCI and CABG in the setting of CS, but the two interventions seem to be comparable in high risk populations.[63] By attacking the culprit lesions only, there is a risk of inadequate revascularization in some of these patients.[64, 65] Potentially, the rather disappointing effects of revascularization could be improved by allocating more patients to early complete revascularization by CABG, as the majority of CS patients present with multi vessel disease.[62]

Less severe forms of acute heart failure have received much less attention than CS in regard to invasive revascularization. Data from the NRMI registry revealed an in-hospital mortality rate of 24 % for this group and invasive revascularization was performed in 20 – 36 % of the patients depending on timing of HF onset.[66] Similar revascularization rates were reported from the GRACE registry in patients with ACS complicated with HF.[67]

Valvular surgery

The rate of severe mitral regurgitation was 12.4 % in the mixed population of patients with cardiogenic shock in EHFS-II, considerably higher than reported from the SHOCK trial registry (7 %).[12, 41] Close to half of the patients with severe MR received valvular surgery in the SHOCK trial registry and these patients had improved unadjusted survival rates compared to conservatively treated patients.[68] The proportion of these patients in which mitral regurgitation was functional, or secondary to left ventricular dysfunction, was not reported. To what extent valve surgery should be performed in the setting of ischemic functional MR and cardiogenic shock, is not known, as revascularization alone can improve cardiac function in these patients. Also, medical stabilisation and IABP support can improve the situation.

Surgical repair can, however, be the only option when conservative treatment fails. For patients presenting with severe aortic regurgitation or aortic stenosis in the setting of cardiogenic shock, the prognosis is particularly bad, and urgent operative valve replacement usually represents the only option for appropriate candidates for surgery.(Katz JN (2009), Valvular heart disease in cardiogenic shock, In J. S. Hochman & E. M. Ohman (Eds.), Cardiogenic shock. Wiley-Blackwell)

Intra-aortic balloon counterpulsation

Despite being a recommended therapy in CS, only 39 % and 18 % in the NRMI registry and EHS ACS 1, respectively, received treatment with IABP.[6, 16, 53, 57, 59] A potential under-utilization of mechanical assistance was also found in EHFS-II where only one-third of patients with CS received such therapy compared to half of the patients in the SHOCK trial registry.[41]

5.5 Limitations to the conventional invasive treatments

Although the aggressive use of early invasive revascularization and IABP has been found to improve long term survival in patients with cardiogenic shock following AMI, the high early mortality in the group is not convincingly reduced.[34] This is a critical shortcoming in the current standard treatment for these patients as the majority of cardiac deaths occur within the first 1-2 days.[17] Additional mechanical circulatory support by means of a ventricular assist device (VAD) represents the only option to ensure survival for some of these patients. VADs are usually applied in refractory circulatory shock when maximal conventional medical and invasive treatments, including the use of IABP, fail to restore the circulation. In this subgroup of patients mortality is exceedingly high, justifying the use of treatment modalities associated with potentially fatal complications and high costs. The reported mortality rates from different

patient series treated with VAD after AMI lies between 30-80 % and can represent enhanced survival for these patients.[48, 69-78] In the setting of AHF, temporary treatment with VADs can serve as a bridge to recovery, long term assist systems or transplantation. It can also facilitate and provide time for patients with the most severely compromised circulation (i.e. cardiac arrest) to undergo necessary revascularization and/or cardiac surgery.[48, 73, 79]

Current guidelines on acute heart failure give a class IIa recommendation for the use of such devices.[6, 55] The robust evidence guiding such treatment is, however, scarce, and not supported by randomized trials.

5.6 The endothelial function during severe acute heart failure

5.6.1 The endothelium and endothelial function in cardiovascular disease.

A healthy vascular endothelium is an active metabolic and endocrine organ with key functions in maintaining vascular homeostasis. It regulates vessel tone by a balanced production of vasodilators (nitric oxide, prostacyclin) and vasoconstrictors (endothelin, thromboxane A_2). It also plays an active role in the equilibrium of hemostasis and fibrinolysis and produce cytokines and adhesion molecules and participate actively in the inflammatory response.[80] Alteration of normal endothelial physiology through an imbalance between vasodilators and vasoconstrictors constitute endothelial dysfunction.[81] Reduced bioavailability of vasodilators, and NO in particular, accounts for the decreased endothelial dependent vascular relaxation – a hallmark of endothelial dysfunction.

Endothelial dysfunction is a systemic process occurring throughout the vasculature and results in abnormal regulation of blood vessel tone and thus regulation of flow and the loss of the atheroprotective properties of the endothelium. Thus, it is viewed a prerequisite for

atherogenesis and development of clinical cardiovascular disease[82]. Importantly, endothelial dysfunction is shown to be predictive of future cardiovascular events[83, 84].

5.6.2 Endothelial function in acute disease states – role of inflammation?

A functional endothelium and nitric oxide bioavailability are essential for maintenance and regulation of blood flow and tissue perfusion. Thus both micro and macrovascular endothelial dysfunction are likely to be key contributors to organ failure in critical disease and sepsis in particular.[85] The role of the endothelium and the significance of endothelial dysfunction during acute disease are not much studied with regards to cardiovascular disease, including acute heart failure. A few clinical studies have assessed vascular reactivity in shock and critical illness, demonstrating an impaired vascular response during reactive hyperemia. Kirschenbaum et al. found an attenuated increase in forearm blood flow during reactive hyperemia in patients with CS and septic shock compared with healthy controls.[86] This finding has been confirmed by measuring microvascular reactivity in both sepsis and septic shock.[87, 88]

Serum from patients with severe heart failure down regulates eNOS and induces apoptosis in endothelial cells, an effect partly caused by TNF-α.[89] During severe decompensation of chronic heart failure it has also been demonstrated that vascular endothelial cells are activated with signs of increased cellular oxidative stress and expression of COX-II and iNOS.[90] A transitory impairment of endothelial function after exposure to cytokines and transitory inflammation has been described in an experimental setting.[91-97] Animal studies have also suggested that TNF-α induce coronary endothelial dysfunction following ischemia/reperfusion injury.[98] This transient effect of inflammation on endothelial function has by some investigators been termed “endothelial stunning”.[97] Furthermore, TNF-α and IL-6 have also been shown to induce endothelium dependent vasoconstriction in human arterial segments and thus the potential predisposition to vasospasm and vessel occlusion.[99]

Both patients with advanced chronic heart failure and acute hear failure have been shown to have elevated circulating levels of inflammatory cytokines[100-103], and TNF-α was related to impaired flow mediated dilation in patients with chronic heart failure[104-106]. Furthermore, experimentally induced heart failure in dogs caused coronary endothelial dysfunction and reduced the aortic expression of eNOS and COX-I.[107] Importantly, treatment with a TNF-α antagonist improved endothelial function in patients with advanced heart failure correlating with a reduction in TNF-α levels, providing a mechanistic link between inflammation and systemic endothelial function.[108] However, large scale clinical trials have failed to show any clinically relevant benefit from anti-inflammatory treatment in cardiovascular disease including heart failure patients.[109-114]

There is evidence that acute systemic inflammation is related to transient increase in the risk of cardiovascular events.[115] Interestingly, Tonetti et al. could demonstrate in a randomized trial that intensive treatment of chronic periodontitis resulted in an acute and transient systemic inflammation accompanied by endothelial dysfunction that resolved with improved endothelial function compared to baseline after 6 months.[116] It has been hypothesized that acute endothelial dysfunction might be the mechanism linking inflammation to acute cardiovascular events.[117]

While most studies focus primarily on the risk for vascular disturbances and cardiovascular events secondary to infection and inflammation, there is increasing awareness on the importance of inflammation and clinically evident SIRS during severe acute cardiovascular diseases such as acute MI and CS.[45, 46, 50, 118-123] Furthermore, elevated levels of inflammatory cytokines are associated with increased mortality in CS and acute MI.[50, 120, 121] The significance and relation of the observed inflammatory response and vascular disturbances in acute cardiovascular disease is, however, unknown.

5.6.3 Role of nitric oxide and endogenous NO inhibition in acute heart failure

Nitric oxide is one of the major endothelium derived vasoactive mediators. The amino acid L-arginin is the substrate for NO synthase (NOS) and thus precursor for formation of nitric oxide (NO). Three isoforms of NOS have been indentified; nNOS (NOS I), iNOS (NOS II) and eNOS (NOS III). Endothelium derived NO produced under basal conditions is stimulated by several receptor agonists and also shear stress induced by blood flow. It is detrimental for vascular homeostasis by regulating vascular tone and blood flow, but acts vasoprotective by inhibiting platelet aggregation, leukocyte adhesion and smooth muscle proliferation.[124] Widespread induction of the inducible form of NOS (iNOS) can be harmful as it leads to vasodilation and hyporeactivity to vasoconstrictors.[52] It may also cause myocardial dysfunction especially in conditions with concurrent oxidative stress.[125] In addition, the formation of reactive nitrogen species (peroxynitrite) can be detrimental for endothelial cells.[126] However, iNOS can have both deleterious and beneficial effects in circulatory shock states and its role in the observed myocardial and vascular abnormalities in CS is still undefined.[125]

The recently published Triumph trial illustrated this complex role of NO in severe heart failure.[127] This trial addressed the possible adverse effects of a presumed excessive nitric oxide production (by iNOS) secondary to inflammation in CS. In this study, treatment with an unselective NO inhibitor in patients with persisting shock failed to reduce the duration of shock and mortality. NO is a principal mediator for refractory hypotension in septic shock[52, 125], but clinical studies using unselective NO inhibitors in sepsis also revealed deleterious effects on organ function and most importantly increased mortality despite positive effects on blood pressure and vascular resistance[128, 129].

Asymmetrical dimethylarginine

Asymmetrical dimethylarginine (ADMA) is a naturally occurring competitive inhibitor of all three forms of nitric oxide synthase.[130] There are several studies suggesting that ADMA is a reliable marker of increased cardiovascular risk. Elevated levels of ADMA have been reported in cardiovascular conditions such as peripheral occlusive arterial disease, stable and unstable coronary artery disease and chronic heart failure.[131] A variety of conditions associated with increased cardiovascular risk such as hypercholesterolemia, hypertension, diabetes mellitus and chronic renal failure have also been reported to have elevated ADMA levels.[131] Importantly, elevated ADMA seems to identify individuals at increased risk for future cardiac events.[132-134] Furthermore, ADMA is also implicated in the pathogenesis and development of endothelial dysfunction.[130, 135-137]

ADMA is formed by methylation of arginine residues of proteins by the action of the enzymes protein arginine methyltransferases (PRMTs) which results in asymmetric dimethylarginine (ADMA), monomethyl arginine (l-NMMA) and symmetric dimethylarginine (SDMA).[138] A subsequent proteolysis of these methylated arginine residues results in free intracellular ADMA that can be transported out of cells by cationic amino acid transporters(CAT).[139] The intracellular concentration of ADMA in endothelial cells is believed to be more than 10-fold higher than circulating levels.[131, 140] Main routes of elimination are metabolic degradation by dimethylarginine dimethylaminohydrolase (DDAH) in the liver and kidney and to a lesser extent by renal excretion.[139, 141] Accumulation of ADMA is though to occur mainly secondary to inhibition and dysregulation of DDAH.[139] DDAH is sensitive to oxidative and nitrosative stress and thus pathogenic stimuli such as inflammation that may lower DDAH activity leading to intracellular ADMA accumulation.[139, 142, 143] Importantly, experimental studies have demonstrated that loss of DDAH activity cause accumulation of ADMA and disruption of NO signalling with subsequent endothelial

dysfunction and increased systemic vascular resistance.[137] However, in vitro stimulation of rat vascular smooth muscle cells with IL-1β up-regulated both iNOS and DDAH[144] and reduced levels of circulating ADMA has been described after experimental endotoxemia and at the onset of acute infection[93, 145]. Of note, the relative contribution of degradation by DDAH and cellular export and import of both L-arginine and ADMA by CAT may vary in different conditions, and thereby affect the intracellular NOS substrate:inhibitor ratio.[140]

In contrast to its increasing recognition as a risk marker in chronic cardiovascular disease, little is known about ADMA's role with regards to acute vascular disturbances in acutely ill patients and acute heart failure in particular. In a relatively large cohort (SHOCK-II trial) of patients with cardiogenic shock following acute myocardial infarction, ADMA was an independent predictor of increased mortality and also associated with increased pulmonary capillary wedge pressure and systolic pulmonary artery pressure.[146] Experimental studies have demonstrated adverse hemodynamic effects after systemic administration of ADMA in healthy humans with reduced heart rate and cardiac output and increased blood pressure and systemic vascular resistance. Furthermore, ADMA impaired the physiologic increase in cardiac output following exercise.[141] Experimental studies have also shown that induction of heart failure in animals lead to increased ADMA levels.[136, 147] In addition, ADMA levels were elevated in patients with chronic heart failure and seemed to further increase in patients with acute decompensation.[148, 149] However, a recent study on acute heart failure patients failed to confirm these findings, and ADMA also did not seem to identify patients with increased risk for future cardiac events including episodes of decompensation.[150]

Vascular and endothelial dysfunction are key components in the development of organ failure and death in critically ill patients. ADMA was an independent predictor for poor outcome and correlated with organ failure in a mixed population of critically ill patients.[151] Also, in septic patients ADMA levels seem to be associated with short term mortality and the

degree of organ dysfunction.[88, 152, 153] The ratio between L-arginine as a substrate for NO-synthesis and ADMA was reduced in sepsis with the lowest L-arginin/ADMA ratio found in patients with shock.[88] Increasing ADMA levels were associated with worsening microvascular reactivity. It has been hypothesized that accumulation of ADMA plays a causal role in the development of organ failure by blocking NO production and thereby reducing organ perfusion.[154] However, others have proposed that accumulation of ADMA secondary to DDAH inhibition is a counter regulatory mechanism to limit excessive NO production in sepsis.[155]

5.7 Circulating endothelial progenitor cells for vascular repair in cardiovascular disease

In 1997 Asahara et al. reported on the existence of circulating bone marrow derived cells that differentiated in vitro into cell with endothelial characteristics. They termed these cells endothelial progenitor cells (EPCs).[156] The potential role for EPCs in preserving a healthy endothelium was further demonstrated by Hill et al. who found a strong correlation between the numbers of colony forming circulating EPCs and conventional cardiovascular risk factors and endothelial function (FMD).[157] A number of studies have demonstrated impaired numbers and function of circulating EPCs in patients with manifest cardiovascular disease[158, 159], but also in conditions associated with increased cardiovascular risk such as diabetes mellitus[160-162], hypercholesterolemia[163], cigarette smoking[164] and chronic renal failure[165]. Also, the numbers of circulating EPCs have been shown to predict future cardiovascular events.[166, 167]

Importantly, these cells have been shown to replenish damaged endothelium and enhance adult neovascularization at sites of ischemia in experimental models.[156, 168-176] Based on these observations it has been assumed that the bone marrow and circulation contains a pool of bone marrow derived endothelial progenitors maintaining vascular homeostasis.

5.7.1 Defining endothelial progenitor cells

Endothelial progenitor cells represent a small subpopulation of peripheral blood mononuclear cells (MNC). Since the first description of EPCs, different techniques for isolation and culture have yielded at least three different putative EPC populations derived from peripheral blood.[157, 177-180] EPCs are most commonly divided into early outgrowth and late outgrowth EPCs with different morphology, functional properties and growth potential. The late outgrowth EPCs differ from early outgrowth EPCs in having a high proliferative capacity and that they do not express the hematopoetic markers CD14 and CD45.[177, 181, 182] It has thus been argued that early outgrowth EPCs consists of myeloid cells of hematopoietic origin.[177, 183] Studies also indicate that early EPCs have a limited degree of engraftment and incorporation into new vessels and it has been proposed that they facilitate vascular regeneration mainly through secretion of proangiogenic factors.[178, 183-186] Late outgrowth EPCs seem to have favourable in vitro angiogenic properties compared to other putative EPCs.[187] Importantly, they have also been demonstrated to form de novo vessels in vivo.[182, 188-190] These studies indicate that late outgrowth EPCs are the true progenitors of mature endothelial cells. However, no studies have so far been performed in humans using this specific cell population.

5.7.2 Therapeutic stem and progenitor cell transplantation

Bone marrow derived stem and progenitor cell transplantation have showed promising effects on reducing infarct size and improving cardiac function in experimental models.[172-174, 191] Despite a modest but beneficial effect on cardiac function and remodelling following cell therapy in clinical trials[192-194], the potent effects observed in experimental models have however not been convincingly reproduced in clinical trials so far. The utility of stem and progenitor cell transplantation is potentially hampered by their low numbers in the circulation, impaired functional activity, induction of unfavourable functional changes during culture and

by inhibitory factors in the recipient causing reduced cell survival and poor engraftment of the transplanted cells.[158, 195-197] Proposed strategies to improve engraftment of transplanted EPCs and bone marrow derived cells include concomitant infusion of proangiogenic cytokines such as VEGF and SDF-1[198, 199], but also by priming cells prior to transplantation[200, 201]. A novel candidate to improve the utility of EPC transplantation is the endogenous vasoactive peptide Adrenomedullin (AM). It is synthesized in most tissues including endothelial cells and has a variety of biological actions suggesting a protective effect against vascular damage and progression of atherosclerosis.[202] The endothelial effects include inhibition of endothelial apoptosis[203] and promotion of endothelial proliferation[204, 205]. Similar beneficial effects of AM have also been demonstrated on early outgrowth EPCs.[206, 207] Of importance, AM has been shown to promote angiogenesis in various experimental models including coadministration with bone marrow derived cells in ischemia.[206, 208-211]

6. Aims of the studies

6.1 Paper I

At the time of planning and initiation of this retrospective study on patients with AHF admitted to the ICU/CCU at the University Hospital of North Norway there were no prior studies describing the overall population of patients with acute heart failure necessitating treatment and surveillance at the ICU/CCU. Of interest, patients with acute heart failure after cardiac surgery, i.e. postcardiotomy HF, have still not been mentioned in the subsequently reported large registries (EHFS II, EFICA, ADHERE).

In this study we give an overview and description of all patients admitted to the ICU/CCU with AHF including patient demographics, prior medical history, precipitating factors for AHF, treatment and outcome. The main purpose was to assess the use of invasive

treatments (i.e. invasive revascularization and valve procedures) in relation to the etiology of AHF and compare its use with existing guideline recommendations. Patients presenting with cardiogenic shock secondary to acute coronary syndromes or valve pathology were individually assessed with regards to missed opportunities for revascularization or valve procedures.

6.2 Paper II

We observed a dismal short term prognosis for patients with the most severe forms of acute heart failure (i.e. cardiogenic shock and postcardiotomy heart failure) in our registry of patients with AHF presented in paper I. These very high early mortality rates have been reported in several publications and remain high despite extensive use of aggressive invasive treatments including revascularization and the use of intra-aortic balloon counterpulsation. An opportunity for improved outcome may lie in early institution of ventricular assist devices (VAD) in selected patients. The aim of this study was to identify the potential candidates for ventricular assist devices in the retrospective patient material presented in paper I and thus estimate the potential need for such therapy in the AHF population. Secondarily, we aimed to compare survivors vs. non-survivors with regards to patient demographics, prior medical history, precipitating factors for AHF and in-hospital treatment in order to identify potential predictors for in-hospital mortality.

6.3 Paper III

This was a pilot study conducted on patients undergoing open-heart surgery (CABG and valve replacement) at our institution. The aim was to study endothelial function (measured as flow mediated dilation) and circulating levels of ADMA and NO metabolites (NOx) during open heart surgery and look for possible relations between the two. Open-heart surgery served as a

controlled setting to observe these parameters from a preoperative baseline through the restitution after the cardiovascular and systemic trauma inflicted by such surgery with cardiopulmonary bypass.

Furthermore, this study served as a preparation for further studies on patients with the most severe acute heart failure presented in paper IV. A similar set of repeated endothelial function assessments and biochemical analyses were planned for both paper III and IV. Importantly, this pilot study could demonstrate if endothelial function measurements using brachial artery ultrasound were applicable in a clinical setting on critically ill patients.

6.4 Paper IV

The current understanding of the pathophysiology that render some patients with acute heart failure with an unfavourable course and lack of response to treatment is limited. This was evident in the recently published Triumph trial that addressed the possible adverse effects of a presumed overt production of nitric oxide secondary to inflammation in CS.[127] Prior observational studies have suggested that systemic inflammation and neurohormonal activation plays an important role in cardiogenic shock.

In this observational study, patients with severe acute heart failure (i.e. cardiogenic shock and postcardiotomy HF) were followed up during the first part of their illness. The aim was to further characterize and try to establish the different factors modulating the circulation in these patients. The main aim was to examine the changes and possible relation between the endogenous NO inhibitor asymmetrical dimethylarginine (ADMA), markers of inflammation and vasodilator function as a measure of vascular and endothelial function during the course of acute heart failure. In addition, we assessed their association with organ dysfunction and patient outcomes.

6.5 Paper V

Our interest and assessment of endothelial progenitor cells (EPC) were motivated by the original "Hill-observation" indicating that cardiovascular prognosis is related to the number of circulating EPCs.[157] However, through our initial work with these colony-forming units grown in endothelial promoting media, it became apparent the true cellular lineage of these units was undefined and not necessarily related to endothelial progenitor or stem cells. Based on the work with these various cell lines, we made some observations clarifying important factors for EPC mediated angiogenesis. For instance, the utility of endothelial progenitor cells transplantation is potentially hampered by their low numbers in the circulation, impaired functional activity and importantly by inhibitory factors in the recipient causing poor cell survival and engraftment. These obstacles can possibly be circumvented by ex vivo priming of harvested cells or co-treatment with proangiogenic cytokines and peptides. In this study we examined the potential role of adrenomedullin to promote growth and angiogenic potential of cultured human late outgrowth EPCs.

7. Materials and Methods

The retrospective review and data collection of patients presented in paper I and II was approved by the Regional Ethics Committee (REK-Nord 51/2004) and the Norwegian Social Science Data Services (ref. 15293). The human observational studies presented in paper III, ,IV and V were approved by the Regional Ethics Committee (REK-Nord 51/2004) and storing of human blood samples were approved by the Norwegian Directorate of Health (Biobank Registry no: 1045). Informed written consent was obtained from all study participants if possible or their nearest relative.

7.1 Retrospective study of patients with acute heart failure (paper I and II)

Data collection

All patients admitted to the ICU or CCU through the years 2003-2004 with evidence of acute heart failure, whether present on admission or later in the hospital stay, were enrolled retrospectively. Eligible patients were screened based on discharge diagnosis of "heart failure", "myocardial infarction" or "unstable angina". Patients with AHF following major non-cardiac surgery (n = 22) were excluded, as for the majority of these patients cardiac dysfunction was secondary to serious underlying surgical conditions and multi organ failure. Data were collected by the investigators from medical records using a case report form that included patient demographics, prior medical history including cardiovascular disease, prior PCI/CABG, renal failure, diabetes and COPD. Precipitating factors and in-hospital invasive treatment were registered as reported. Results of laboratory tests, chest x-ray, echocardiography and clinical tests were all collected after the onset of AHF. Angiographic data referred were the most recent findings from the index hospitalisation. Data on in-hospital and two year mortality were obtained in 100% of the cases by matching all patients with the Norwegian Cause of Death Registry.

Acute heart failure classification

Patients were classified by the investigators into the following AHF conditions based on the current ESC guidelines[6, 12] : Cardiogenic shock, Acute decompensated HF, Pulmonary oedema, Hypertensive AHF and Right HF. Less severe AHF was defined as all AHF conditions except CS and postcardiotomy HF. Description of clinical symptoms and signs (dyspnoea, rales, hypotension, hypoperfusion, peripheral oedema, increased jugular venous pressure and liver size), X-ray results and attending physician's diagnosis were used for

verification of heart failure and classification into different clinical conditions on admission to the ICU/CCU. Patients with primarily infection, septic shock or other forms of high output failure were excluded.

Postcardiotomy HF is not described in the ESC guidelines. We included patients with inadequate cardiac performance after surgery in need of inotropic and/or mechanical support lasting more than 2 hours.

Evaluation of invasive treatment

In order to clarify whether patients with acute heart failure received invasive treatment according to relevant guidelines, patients with AHF following ACS and/or severe valvular pathology were assessed for type of invasive treatment given. Patients with CS not receiving recommended invasive treatment where reviewed separately to assess eligibility for invasive treatment. Obvious contra indications included death occurring before intervention was possible, evidence of severe irreversible organ damage or the presence of concomitant severe life limiting disease.

Identification of potential candidates for ventricular assist devices

In order to assess the potential candidates for treatment with ventricular assist devices, non-survivors with the most severe forms of acute heart failure, i.e. CS and postcardiotomy HF, were individually reviewed. Eligible patients were defined as all patients dying during hospitalisation despite maximal conventional treatment efforts. Secondarily, exclusion criteria were age above 75 years, pre-existing severe chronic heart failure, comorbidities with reduced life expectancy, severe peripheral vascular disease and evidence of permanent end organ damage or multi organ failure before institution of VAD treatment was considered feasible.

7.2 Endothelial function measurements

There are several techniques for measurement of endothelial function.[81] The "gold standard" is invasive measurement of change in coronary artery diameter, blood flow and vascular resistance secondary to intra coronary infusion of acetylcholine.[212] As a substitute for direct measurements of coronary vascular reactivity there are several non invasive techniques measuring peripheral vascular reactivity to reflect systemic changes in endothelial function including the coronary arteries. The most widely used non invasive technique is ultrasound measurements of brachial artery dilation.[213] This method measures the physiologic dilation of the brachial artery (conduit artery) induced by increased flow and shear stress acting on the endothelium. The magnitude of this vasodilatory response serves as an index of endothelial function and is referred to as flow mediated dilation (FMD). Another emerging method for measurement of peripheral vascular reactivity is the use of fingertip pulse amplitude tonometry using a finger plethysmograph.[214] As opposed to FMD, a measure of conduit artery dilation, this method measures the microvascular vasodilator response in the fingertip. Although both techniques have been shown to correlate with several traditional cardiovascular risk factors and the two methods have some degree of agreement, some observations suggest that they do not interchangeably measure an equal vasculatory response.[214-216] However, both methods have been shown to reflect NO-bioavailability.[217, 218] Both techniques measure the distal vascular response to a transitory arterial occlusion resulting in local ischemia and subsequent reactive hyperemia when blood flow is re-established. The reactive hyperemia is a complex process involving metabolic vascular responses, myogenic responses, neuronal responses and endothelium dependent responses.[219, 220] The principal mediators for the endothelium dependent dilation are NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF). FMD measured in a strictly controlled setting is thought to be NO dependent and reflecting NO bioavailability. However, it is debated to what degree flow-

mediated dilatation reflects NO mediated dilation.[221-223] The relative importance of NO as opposed to other endothelial derived mediators seems to depend on methodology i.e level of occlusion, type of artery (vessel size), occlusion time and patient related factors (CAD- vs healthy).

Brachial artery ultrasound measurements

Bracial artery ultrasound imaging was used to measure flow mediated dilation in paper III. The diameter of the brachial artery was measured 5-10 cm proximal to the antecubital fossa using a Phillips iE33 ultrasound machine with a vascular probe (11 MHz), held in position by a stereotactic clamp after optimal visualization of the lumen-vessel wall interface. The vessels were scanned longitudinally using a 2D greyscale image and diameters were measured at end diastole. Reactive hyperemia was induced by occlusion with a blood pressure cuff on the proximal forearm and inflated to minimum 200 mmHg and at least 50 mmHg above systolic blood pressure for 5 minutes. Digital images were recorded at baseline and then continuously for 2.5 minutes during reactive hyperemia. Pulsed wave Doppler measurements were obtained within 15 seconds after deflation. A second resting baseline scan was performed 15 minutes after the first one. Images were analyzed using commercially available software with automatic edge detection of a selected segment of the artery (Brachial analyzer, Vascular research Tools 5.0.4, Medical Imaging Applications LCC, USA). FMD was expressed as the relative change (%) in vessel diameter after 60 seconds of hyperemia compared to baseline diameter. Flow was calculated from total averaging mean velocity (TAMV) measured at baseline and immediately after cuff release. Reactive hyperemia was calculated as hyperaemic flow increase relative to baseline.

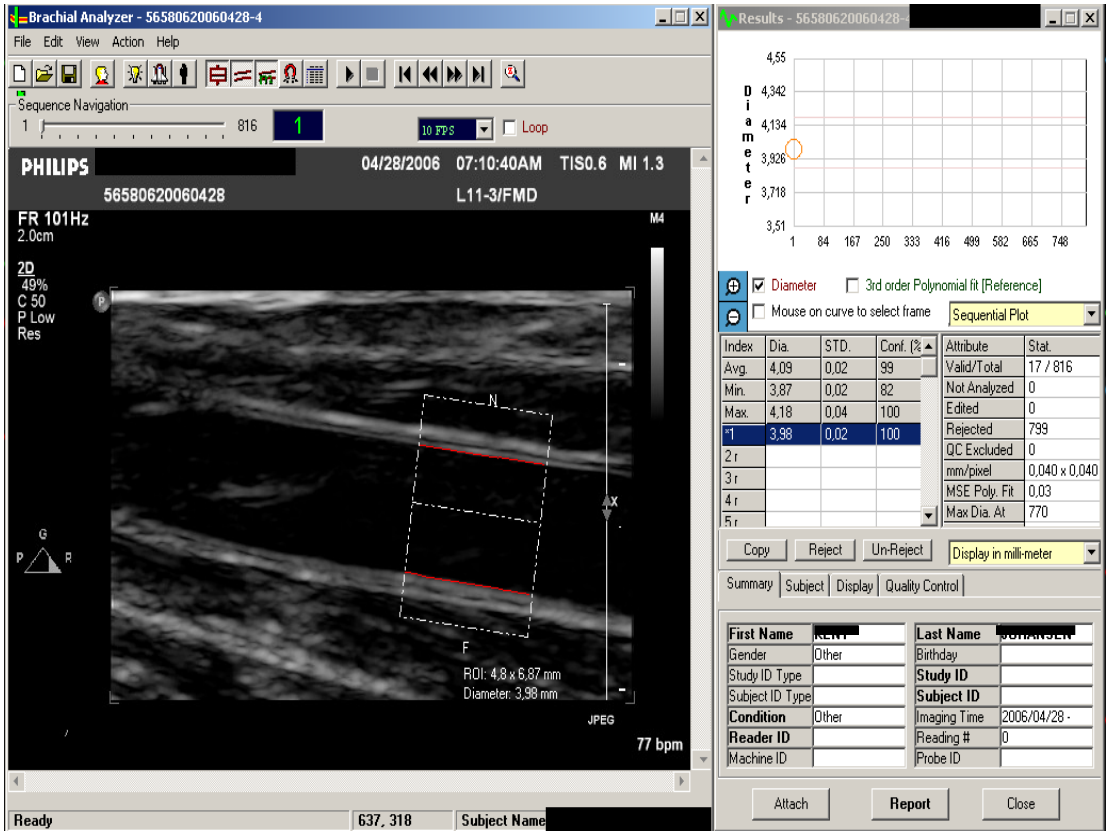


Fig 1. Example of automatic edge detection and measurement of brachial artery diameter using the Brachial analyzer software on 2D grey scale ultrasound images.

This technique has some limitations and requires skilled and experienced operators and is clearly user dependent. Being the most used and validated technique, FMD, was used to assess vascular function in paper III. It was, however, apparent that it was difficult to adhere to the proposed recommendations for standardisation of FMD measurements in an intensive care setting and in the postoperative care with limited patient's cooperation.[213] For studying patients with acute heart failure the use of EndoPAT proved more feasible and had the advantage of being user independent.

Peripheral arterial tonus measurements (EndoPAT)

Endothelial function measurements presented in paper VI were assessed with non invasive digital pulse amplitude tonometry using the EndoPAT 2000 (Itamar Medical LTD, Caesarea,

Israel). This device provides measurements of the vasodilator function during reactive hyperemia with a fingertip plethysmograph measuring pulsatile volume changes in digital microvessels reflecting the peripheral arterial tone (fig 2.).

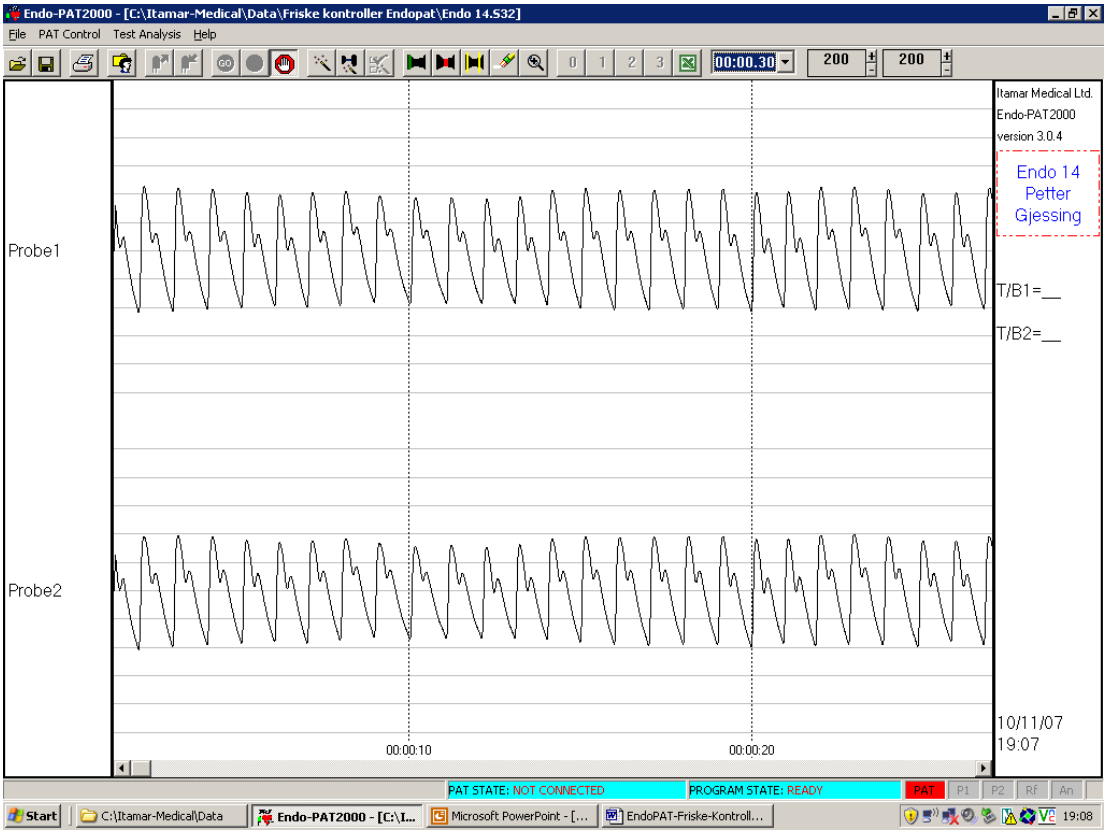


Figure 2. Image shows baseline recordings of the pulsatile blood volume changes in the fingertip using the EndoPAT 2000.

Measurements were performed bedside with patients in a supine position. Reactive hyperemia was induced by a 5 min. occlusion of the upper arm at approximately 60 mmHg above systolic blood pressure. Both sides were measured simultaneously to allow for adjustments of systemic changes in arterial tone by correcting for changes in the non occluded arm. Results were analysed using automated analysis software supplied by the producer (EndoPAT 2000

software version 3.1.2). This software calculates the ratio between baseline and hyperaemic pulsatile volume changes presented as a reactive hyperemia index (RH-index).

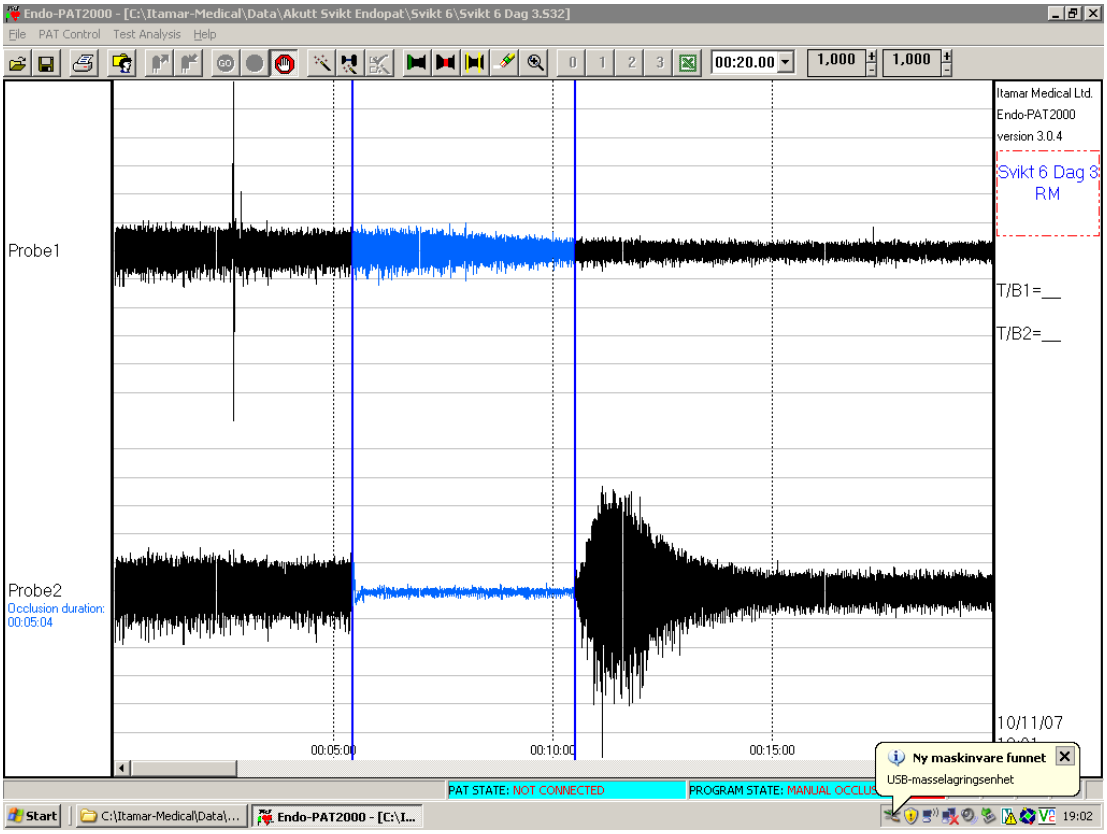


Figure 3. Example of a normal hyperemic response recorded in a patient with acute heart failure using EndoPat 2000. The blue segment marks the 5 min. occlusion period also seen by absence of signal in the occluded arm. The image shows a normal hyperemic response in a patient with acute heart failure.

7.3 Endothelial progenitor cells and angiogenesis

7.3.1 Cell isolation and culture

Human endothelial progenitor cells were isolated from both patients undergoing open-heart surgery (from paper III) and healthy voluntaries (paper V). It was originally planned to use cells from patients for studies on late outgrowth EPCs and angiogenesis. In patients undergoing surgery with manifest coronary heart disease we were, however, not able to

culture late outgrowth EPCs, probably due to the low number of cells in the circulation. We therefore used blood from healthy voluntaries for the experiments presented in paper V.

Early outgrowth endothelial progenitor cells

Blood samples from patients were isolated and cultured based on a previously described method.[168] In short, mononuclear cells (MNC) were isolated by density gradient centrifugation, suspended in endothelial growth media (EGM-2, Bulletkit, Cambrex Bio Science) and cultured on 4-well culture slides coated with humane fibronectine. Non-adherent cells were removed on day 4 and cultures maintained until day 7 before staining. Cells were stained by incubation with Dil-labeled acetylated LDL and FITC conjugated lectin (Ulex Europaeus, UEA-1). Cells with endothelial phenotype positive for both lectin and Dil-ac-LDL were judged as early outgrowth EPCs and enumerated using an inverted fluorescence microscope (fig 4).

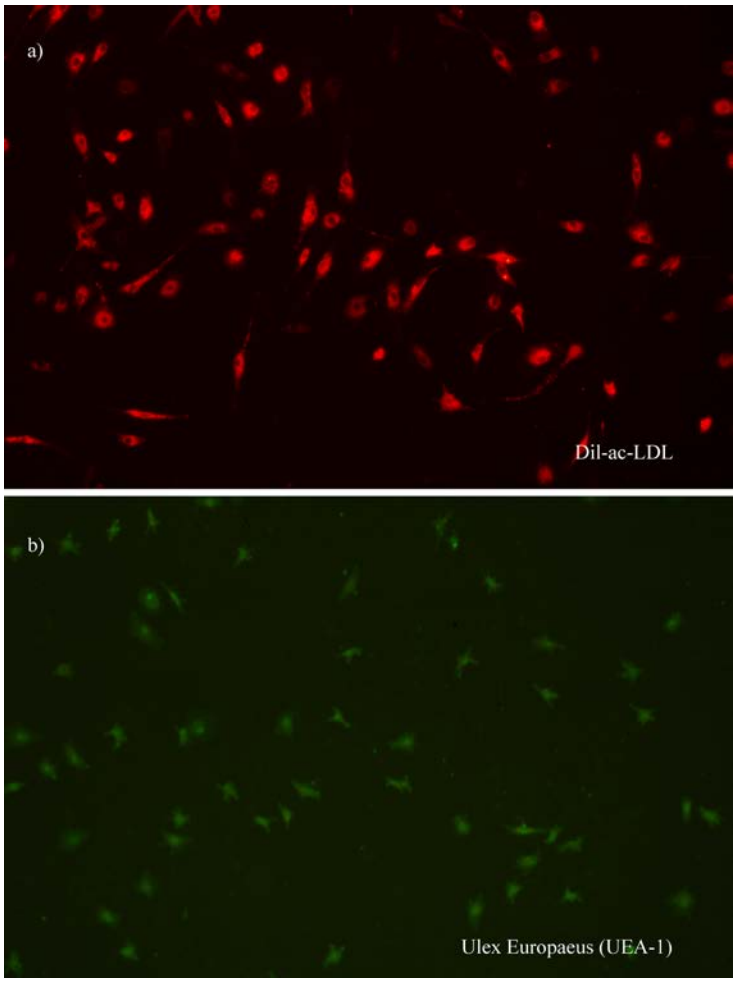


Figure 4. Image of cultured early EPCs. a) Stained with Dil-labelled (red fluorescence) acetylated LDL. b) Stained with FITC-labelled (green fluorescence) Ulex Europaeus lectin (UEA-1).

Late outgrowth endothelial progenitor cells

Human mononuclear cells from freshly collected blood were isolated using density gradient centrifugation. The cells were suspended in endothelial growth media (EGM-2, Bulletkit, Cambrex Bio Science) and seeded at 5 x 10^6 cells/well onto six-well plates precoated with rat tail collagen type 1. Non-adherent cells were removed after 24 hours. Cultures were maintained until the appearance of typical late outgrowth EPC colonies with cobblestone morphology (for a maximum of three weeks) (fig 5). Cells in passage 3-8 were used in all experiments.

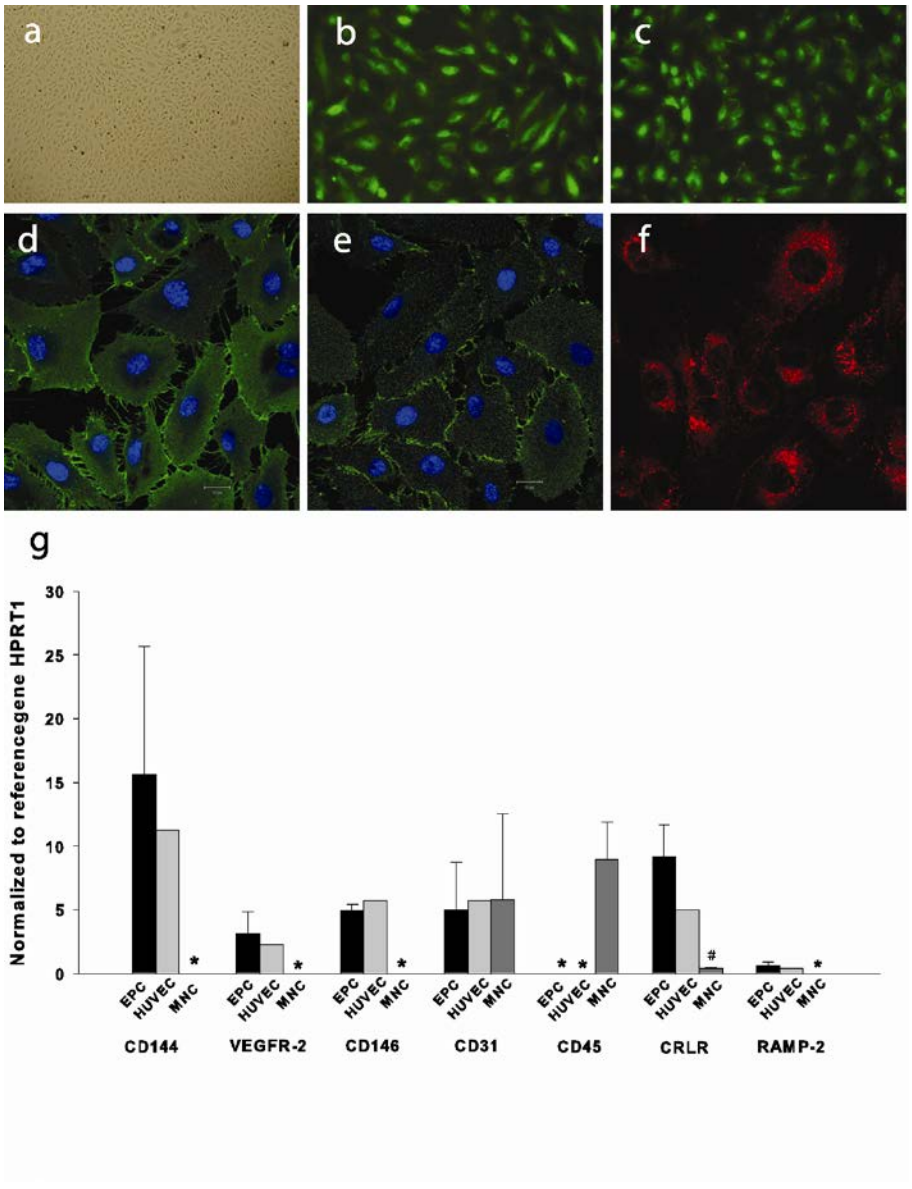


Fig 5. Phenotypic characterization of late outgrowth endothelial progenitor cells (paper V). a) Confluent layer of EPCs in culture. b) and c): Immunofluorescence staining: Receptor activity modifying protein 2 (RAMP-2) (b) and calcitonin recepror-like receptor (CRLR) (c). d) and e): Confocal photomicrographs: platelet endothelial cell adhesion molecule (PECAM, CD31) (d) and VE-cadherin (CD144) (e). Cell nuclei are counterstained with Draq5 (blue). f) Uptake of Dil-Ac-LDL. g) Real-time PCR results of the relative mRNA expression of relevant genes to characterize EPCs compared with human umbilical vein endothelial cells (HUVEC) and mononuclear cells (MNC). * No expression. # p = 0.001 compared with EPC. VEGFR-2, vascular endothelial growth factor receptor 2.

7.3.2 In vitro proliferation and angiogenesis assay

EPC proliferation was assessed using CELLTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega), a colorimetric determination of viable cells based on the bioreduction of a tetrazolinum compound (MTS) in metabolically active cells.

To assess the angiogenic properties of EPCs we used a previously described coculture assay.[187, 224] Human fibroblasts (MRC5) were seeded onto a 24-well culture plate and allowed to grow to confluence. EPCs (1×10^4) were added and cocultures were grown for 14 days in EGM-2 (without VEGF) with the addition of either adrenomedullin (AM) or vascular endothelial growth factor (VEGF). Finally, cocultures were fixated and stained with CD31. Tubular networks were photographed with an inverted fluorescence microscope. In addition, we used a short-lasting (18 h) angiogenesis assay with 24-well culture plates coated with Growth Factor Reduced Matrigel Matrix (BD Biosciences). Culture plates were photographed using an inverted light microscope. The extent of tubular networks in both assays were analyzed using Scion Image software.

8. Summary of results and discussion

8.1 Summary of results Paper I

A total of 324 patients diagnosed with acute heart failure were treated at the CCU and/or ICU during the two year observation period. The timing and location of AHF onset is shown in figure 6. The majority of patients (62 %) presented with signs of acute heart failure on admission to the hospital, while 25 % occurred after intervention. The distribution of the different clinical AHF conditions is shown in figure 7.

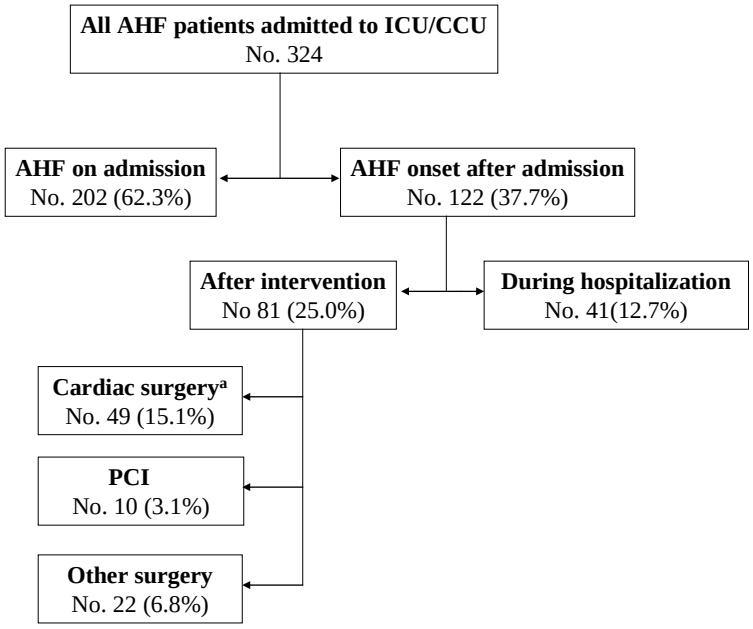


Figure 6. Timing of acute heart failure (AHF) onset. a) Includes also patients without postcardiotomy HF necessitating treatment at CCU/ICU. CCU, Coronary care unit. ICU, Intensive care unit. PCI, Percutaneous coronary intervention.

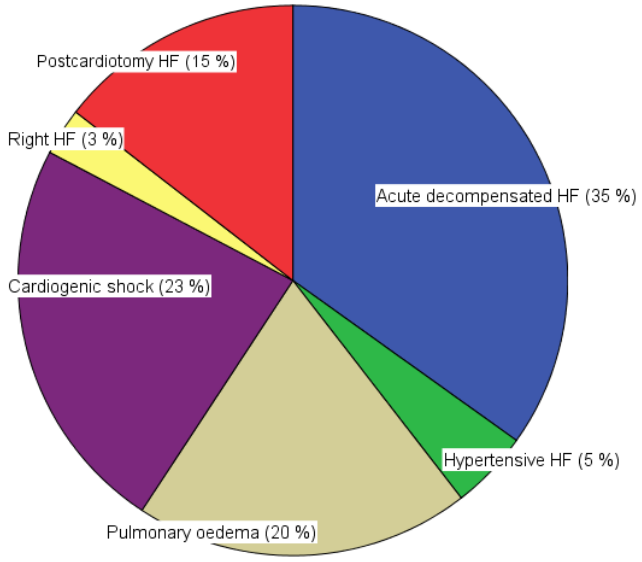


Figure 7. Distribution of the different acute heart failure conditions admitted to the intensive care unit/coronary care unit at the University Hospital of North Norway 2003-4.

Less severe forms of AHF (i.e acute decompensated heart failure, pulmonary edema, hypertensive acute heart failure and right heart failure) accounted for 62 % of the patients. The most severe forms of AHF, CS and postcardiotomy heart failure were seen in 23 and 15 % of the patients, respectively. CS patients were on average younger (69 years) compared with less severe forms of AHF (75 years). Cardiovascular comorbitities were abundant in the overall population with half of the patients presenting with a history of coronary heart disease and 40 % had prior myocardial infarctions. Prior invasive revascularization, PCI (12 %) and CABG (14%), had been done in one-fourth of the patients. Acute coronary syndromes were by far the most common precipitating factors for acute heart failure (57 %). The vast majority of these patients had evidence of acute myocardial infarction. A large proportion, 30 %, presented with arrhythmias usually concomitant to other precipitating factors. Severe valvular dysfunction was observed after acute heart failure onset and considered as a precipitating factor in 12 % of the overall population and in 20 % of CS patients.

Angiography was performed during the index hospitalisation in 42 % of all patients and most frequently in CS patients (70 %). Three-vessel disease was present in 40 % of the angiograms. The majority of patients had a normal or moderately reduced left ventricular ejection fraction regardless of clinical class. Creatinine levels increased on average for all groups during hospitalization. Hyponatremia was present in 9 % on AHF onset, while

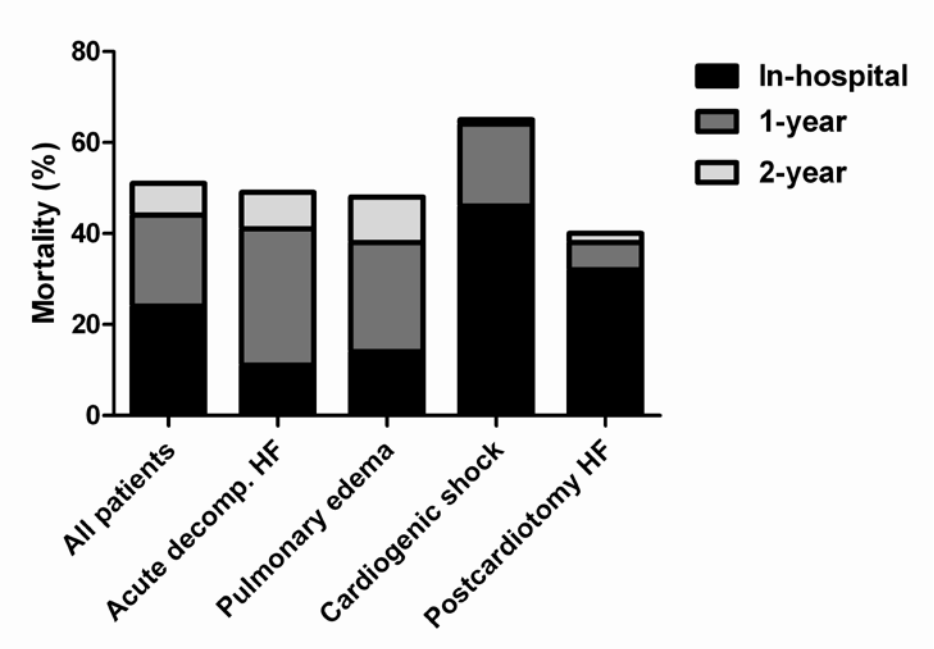
abnormal serum potassium was present in 37 %. Biochemical markers of cardiac injury were significantly higher in CS patients compared to less severe forms of AHF, but not compared to postcardiotomy HF and right HF.

Mortality

The overall in-hospital mortality was 24 %. There was no major difference in mortality between AHF secondary to acute coronary syndromes versus the overall mortality for AHF. CS had the worst in-hospital morality (46 %) and the majority of fatalities occurred within two days after admission. In comparison, less severe forms of AHF had a far better short-term prognosis with an in-hospital mortality rate of 13 %. Two-year survival was worse for CS compared to postcariotomy HF (Log-Rank $p = 0.004$) and other less severe AHF conditions (Log-Rank $p < 0.001$), while no difference was observed between postcardiotomy HF and less severe AHF (Log-Rank $p = 0.064$). Two-year mortality rates for hospital survivors were similar when comparing CS and less severe AHF conditions ($p = 0.709$), while a significantly better two-year survival was observed for the discharged postcardiotomy HF patients ($p = 0.009$). Mortality rates by the major AHF conditions are shown in figure 8a. Two-year Kaplan-Meier survival curves are shown in figure 8b.

Invasively treated patients (revascularization, valvular surgery and other acute cardiac surgery) in the overall population with less severe AHF had lower in-hospital mortality compared to patients treated conservatively (6 % vs. 17 %, $p = 0.042$). This was similar in the subset of patients with acute coronary syndromes (6 % vs. 20 %, $p = 0.028$). Furthermore, CS patients treated invasively had improved outcome both in the overall population (35 % vs 70 %, $p = 0.006$) and when CS was secondary to ACS (34 % vs. 80 %, $p = 0.009$). There was no statistically significant difference in mortality between CS patients treated with CABG versus PCI (15 % vs. 43 %, $p = 0.122$).

a



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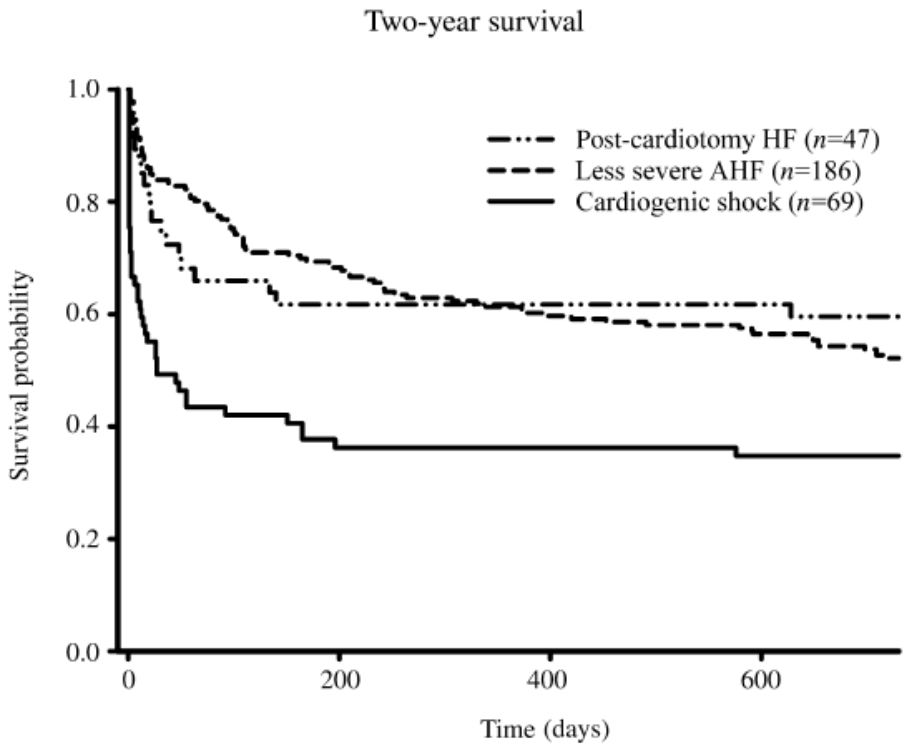

Figure 7. a. All-cause mortality rates by clinical class. b. Kaplan-Meier plot of two-year survival.

Invasive treatment of acute heart failure following acute coronary syndromes

Angiographic examination was performed during hospitalisation in 68 % of all patients with ACS, while 65 % received invasive revascularization. PCI was the treatment of choice in most patients (48 %) and CAGB was performed in 17 %. One patient with CS received both. Revascularization was more frequent among CS patients compared to other less severe AHF conditions (78 % vs. 58 %, p = 0.012). Mechanical circulatory support was used in 22 % of all patients with ACS. CS and postcardiotomy HF patients were treated with IABP in respectively 45 % and 70 % of the cases. CS patients also received mechanical ventilation (intubation) in a majority of the cases (59 %). Cardiac surgery for mechanical complications following ACS, such as rupture of the ventricular septum or free wall, acute mitral regurgitation, cardiac tamponade or concomitant chronic valvular pathology, was performed in 8 %. Most of these procedures were done in combination with surgical revascularization.

PCI and/or CABG were not performed in 11 (22 %) of CS patients with ACS during this first part of their illness. An individual review revealed that only four of these patients (8 %) were untreated potential candidates for coronary angiography and invasive treatment. None of these patients received thrombolytic therapy. All were octogenarians with a history of MI or prior invasive revacularization and none survived the index hospitalisation. In addition, two patients were discharged alive after successful medical reperfusion confirmed by angiography. The presence of severe comorbidities was the reason for not performing angiography in those patients where this was technically feasible.

Valvular pathology

Mitral valve dysfunction was the most abundant valvular pathology and moderate or severe mitral regurgitation (MR) was present in almost one-third of all the patients. Severe MR was observed in 13 % (n = 8) of CS patients and seven of these patients had acute myocardial

infarction. Only one patient received urgent surgery due to a ruptured papillary muscle while the remaining patients were revacularized or treated conservatively. Three patients presented with severe aortic stenosis (AS). In two patients CS was primarily due to worsening AS and they received acute aortic valve replacement. One patient had concomitant AMI and was revascularized. Three patients presented with severe aortic regurgitation. Valvular dysfunction was the main precipitating factor for CS in two patients and one received valve replacement while the other was treated conservatively due to high age and severe comorbidities. One patient presented with concomitant AMI and was revascularized without any valvular surgery.

In the less severe AHF conditions, severe MR was observed in 6 % (n = 10) while severe aortic valve dysfunction was observed in 8 % (n = 13) of the examined patients. Valvular surgery was performed during index hospitalisation in only three patients with severe MR due to rupture of the papillary muscle and endocarditis.

8.2 Summary of results Paper II

Severe acute heart failure was present in 42 % of the over all population with acute heart failure (cardiogenic shock 23 %, postcardiotomy HF 19 %). 18 % of patients with postcardiotomy HF had some form of less severe heart failure before surgical treatment.

Survivors in the cardiogenic shock group were younger (64 vs. 75 years) and were less likely to have a history of coronary heart disease (24 % vs. 47 %), hypertension (19 % vs. 50 %) and peripheral vascular disease (0 % vs. 16 %). There were no major differences between survivors and non-survivors in the postcardiotomy HF group with regards to prior medical history and patients demographics. There was, however, a significantly higher proportion of patients with AHF secondary to valve replacement surgery among non-survivors. Cardiogenic shock survivors were treated more invasively with CABG (32 % vs. 6 %), mechanical

ventilation (70 % vs. 34 %) and IABP (57 % vs. 28 %) compared to non-survivors. Treatment with PCI (41 % vs. 46 %) was equal in the two groups.

In a multivariate analysis, valvular surgery, female gender and high creatinine levels were independently associated with adverse outcome for patients with postcardiotomy HF. For cardiogenic shock patients a history of hypertension and advanced age was associated with increased risk, while undergoing CABG during hospitalisation was associated with increased survival.

Among non-survivors, 27 patients were younger than 75 years. Serious comorbidities and/or evidence of end-organ damage were present in 12 patients, making them unsuitable for treatment with ventricular assist devices. The remaining 15 patients were deemed eligible for VAD treatment and they accounted for 14 and 9 % of patients with CS and postcardiotomy HF, respectively. Nine of these patients presented with CS secondary to AMI complicated with refractory cardiogenic shock after treatment with an early revascularization strategy.

These figures indicate that mortality of potential VAD candidates on the indication acute heart failure is 15/million inhabitants/year.

8.3 Discussion paper I and II

Invasive treatment and mortality in cardiogenic shock

In our cohort of patients, there was an extensive use of invasive revascularization for patients with cardiogenic shock following ACS. Appropriate selection of CS patients for invasive revascularization was confirmed by an individual review of cases receiving conservative treatment. These patients were octogenarians with partly severe comobidities or previous revascularization attempts, and three out of four had non-STEMI. These aspects are likely to

have affected the decision towards conservative treatment and thus indicate an appropriate clinical selection of patients for invasive treatment.

A revascularization frequency of 78 % for CS patients in our survey was substantially higher than reported from EHFS-II and the GRACE registry.[12, 56] We did not, however, observe a more satisfactory in-hospital outcome compared to the EHFS-II. One possible explanation could be that aggressive revascularization is associated with increased early mortality compared to conservative treatment and that the beneficial effects are seen later on, as was the case in the SHOCK trial. In this trial, patients receiving early revascularization had a high early mortality rate of 47 %.[225] Mortality rates for CS of any cause in our material were comparable with the early and long term mortality rates found in the French EFICA study and another published study from Rudiger et al. probably reflecting a similar patient selection.[7, 11] Importantly, survivors of the initial event among CS patients had a relatively good long-term prognosis comparable to other less severe forms of AHF.[11, 226-228]

Compared to other patient materials, the use of surgery (24 %) was high in CS secondary to ACS.[12, 16, 56] In our survey, patients who were revascularized by means of CABG had an 85 % in-hospital survival compared to 57 % in patients treated with PCI, but this was not statistically significant in our relatively small patient cohort. Also, different patient selection and timing of the two interventions could affect these figures.

Valvular pathology was quite common in CS and the rate of severe mitral regurgitation (13 %) was comparable with the findings in EHFS-II, but higher than reported from the SHOCK trial registry (7 %).[41] In the majority, MR was secondary to left ventricular dysfunction and treated with revacularization or conservative treatment only. In contrast, close to half of the patients with severe MR in the SHOCK trial registry received valvular surgery.[68] A different incidence of the two conditions could potentially explain both the occurrence and management of these patients. In addition, there is no definite evidence

guiding the choice between revascularization and stabilisation versus prompt valve repair or replacement when MR follows left ventricular dilatation in acute heart failure. Patients with aortic valve dysfunction received valve replacement when this was technically feasible and there were no major contraindications.

The use of intra-aortic balloon counterpulsation (45 %) was surprisingly low despite being a recommended therapy for CS in particular. Although our figures were better than reported from the NRMI registry (39 %) and EHFS-II (31 %), IABP was used more frequently in the SHOCK trial registry (53 %).[12, 16, 41] This revealed a potential under-utilization of mechanical assistance and since this survey, we have instituted IABP as a routine modality in CS.

Invasive treatment and mortality in less severe acute heart failure

A substantially higher frequency of invasive revascularization was observed in our material (58 %) compared to the NRMI and GRACE registries.[66, 67] Despite similar in-hospital mortality rates on 14 %, the reported long term survival in the GRACE registry was better. This reflects the strikingly high intermediate-term mortality for hospital survivors observed in our patients. A selection of CCU/ICU patients and not the general AHF population, could explain this difference. Mortality rates for less severe forms of AHF of any cause were also a lot higher than reported in EHFS-II and other studies [7, 10, 12], but comparable to the findings in the EFICA study on ICU/CCU patients [11].

Severe valvular dysfunction was less frequent in less severe AHF compared to CS, mostly due to a difference in the presence of severe mitral regurgitation. There was however, no major difference in the frequency of valvular surgery in this group compared to cardiogenic shock, and surgical treatment of acute mitral regurgitation was only performed in the setting of acute papillary muscle dysfunction or rupture and endocarditis.

The dismal long term prognosis for patients with less severe forms of AHF underlines the need for close surveillance after discharge in this CCU/ICU treated population.

Postcardiotomy HF

Postcardiotomy HF constituted a significant part of the total AHF population treated in the CCU/ICU. Short term prognosis was dismal compared to less severe forms of AHF, but better than for CS. Two-year mortality for hospital survivors was, however, substantially better compared to any of the other AHF conditions. These patients are all treated with complete surgical correction, and for logistical reasons, always receive a high level of medical attention and surveillance, making early application of appropriate therapy possible. The extent of myocardial damage was comparable to CS as judged by peak CK-mb.

Candidates for ventricular assist devices

The utilization of ventricular assist devices in the setting of acute CS is not well established. In the SHOCK Trial Registry only 0.8 % of the patients received this treatment. [41] A substantially higher use of VAD therapy (12 %) was, however, reported in the more recent TRIUMPH Trial on patients with CS persisting after revascularization.[127] Tayara et al. reported similar figures on VAD use (13 %) on a series of consecutive patients with CS after AMI.[75] Our estimate of potential candidates for VAD therapy on 14 % in CS is in agreement with these publications.

The use of VADs in the setting of postcardiotomy HF is a more widespread and accepted therapeutic intervention. In the US "Society of Thoracic Surgeons' National Cardiac Database" this intervention was used in 0.3 % of all open heart operations with a reported operative short term mortality on 40 %.[30] Others have reported similar figures on the incidence of severe postcardiotomy HF requiring VAD therapy ranging from 0.2-1.2 %.[27-29,

[31-33] The estimated need for VAD therapy on 0.4 % of all open-heart operations in our survey is well within the range of these reports.

As expected, older patients were overrepresented among non-survivors with cardiogenic shock and advanced age was independently associated with increased mortality. This was not evident in postcardiotomy HF and this could possibly be due to a selection of patient scheduled for open heart surgery based on a total risk assessment with the result that they are otherwise healthier compared to their younger counterparts. A prominent feature in postcardiotomy HF was a substantially increased risk for females and patients undergoing valvular procedures. Female gender is a well established risk factor in open-heart surgery. All the potential VAD candidates in our material with postcardiotomy HF underwent aortic valve replacement surgery with or without concomitant coronary revascularization. This reflects the overall increased risk of perioperative complications following valve procedures compared to isolated coronary revascularization. However, in the largest registry to this date VAD therapy was by far most frequently applied after isolated coronary revascularization.[30]

Concluding remarks

The most severe forms of acute heart failure, i.e. cardiogenic shock and postcardiotomy HF, include a spectrum of patients with different prognosis and degrees of hemodynamic compromise. This heterogeneity and the extremely high mortality in subgroups of these patients mandates an effort to develop more precise diagnostic algorithms to grade the most severe forms of heart failure to ensure a timely and proper selection of patients that can benefit from prompt circulatory assistance.

8.4 Summary of results Paper III

In this study we included a total of 27 patients scheduled for standard coronary bypass surgery (CABG, n = 22) or isolated valve procedures (n = 5). This study also served as a pilot study for the planned study on patients with severe acute heart failure using a similar design. All patients were assessed preoperatively (baseline) and the following three postoperative days with repeated measurements of endothelial function (flow mediated dilatation, FMD) and determination of circulating levels of NOx and asymmetric dimethylarginine (ADMA).

Open heart surgery significantly affected the endothelial function with a marked attenuation in FMD on the first postoperative day compared to baseline (4.1 % vs. 1.4 %, $p < 0.001$). This effect was only transient as FMD returned to baseline levels on the second and third postoperative day. This is shown in figure 9.

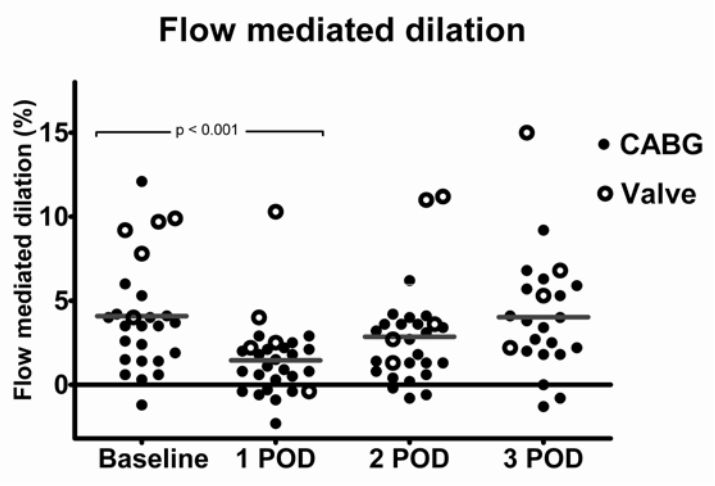

Figure 9. Flow mediated dilation during open-heart surgery (n = 25). Lines denote mean values. POD, Postoperative day.

Patients undergoing isolated valve surgery did not have any evidence of coronary heart disease on coronary angiography. As expected their baseline FMD was higher than for CABG patients (8.1 % vs. 3.1 %, $p = 0.005$). A similar attenuation of FMD was, however, evident in both groups when analysed seperatively (valve surgery: 8.1 % vs. 3.7 %, $p = 0.026$, CABG: 3.1 % vs. 0.9 %, $p < 0.002$).

Open heart surgery affected circulating ADMA levels uniformly with significantly lower levels postoperatively compared to baseline (Friedmans ANOVA χ^2 = 29.3, $p < 0.001$). Postoperative ADMA levels remained significantly suppressed compared to preoperative levels at all the measured time points after correction for multiple comparisons ($p \leq 0.002$, Figure 10a). Surgery also suppressed plasma NOx levels (n = 27, Friedmans ANOVA χ^2 = 24.1, $p < 0.001$). Reduced postoperative NOx were found at all measured time points after correction for multiple comparisons ($p \leq 0.05$, Figure 10b).

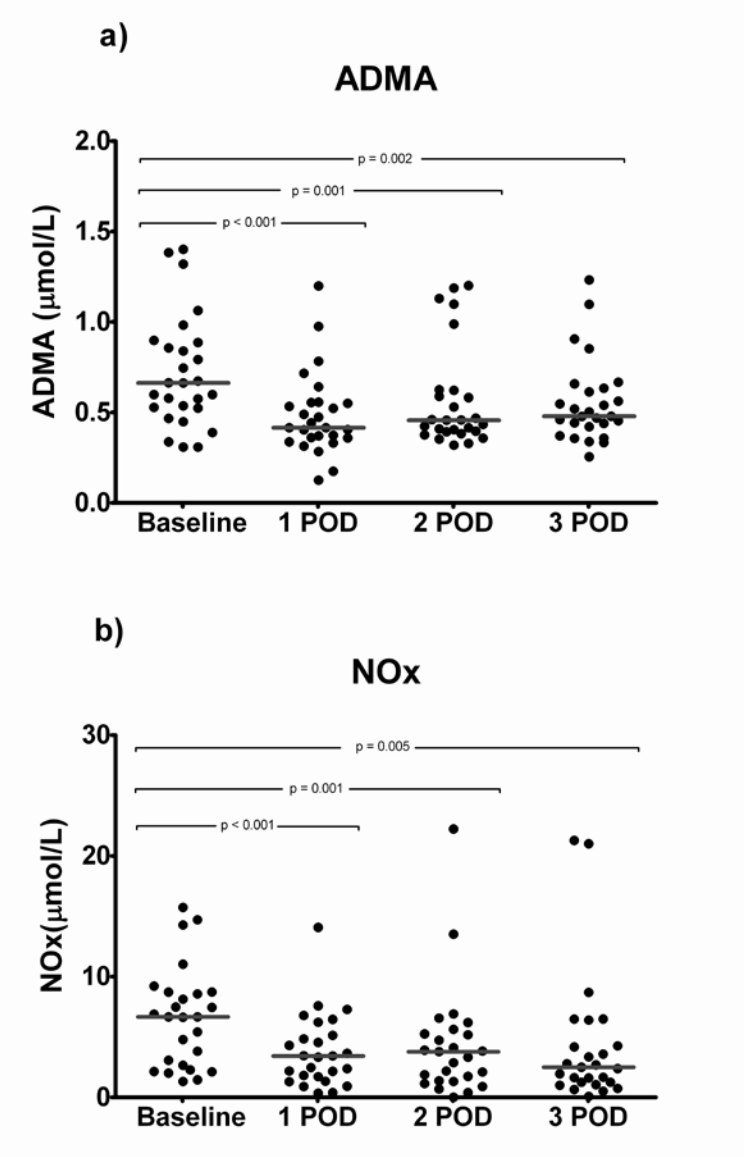

Figure 10. ADMA (asymmetric dimethylarginine) and NOx (nitric oxide metabolites) during open-heart surgery (n = 27). Lines denote median values. POD, Postoperative day.

FMD measurements did not correlate with ADMA/NOx levels at any of the measured time points. Baseline levels of ADMA and NOx did not correlate, but there was a significant positive correlation between NOx on the first postoperative day and ADMA levels on baseline and the first postoperative day ($r = 0.46$, $p = 0.016$, $r = 0.45$, $p = 0.020$). Repeating the analysis with NOx indexed to creatinine did not change this relation.

8.5 Summary of results Paper IV

The study population consisted of 24 prospectively included patients admitted with either cardiogenic shock or postcardiotomy HF at the University Hospital of North Norway between 2007 and 2009. After inclusion, patients were followed with repeated assessment of peripheral vasodilator function, markers of inflammation and endothelial activation, and the endogenous NO inhibitor ADMA.

In-hospital mortality rates were 42 % and 17 % for respectively CS and postcardiotomy HF patients. A marked elevation in the inflammatory markers IL-6 and 8 in the first 1-2 days was a prominent feature in both groups.

The serial assessment of peripheral vasodilator function is shown in figure 11. RH-index was impaired at baseline in both groups (CS: 1.35, $P = 0.001$, Postcardiotomy HF: 1.45, $P = 0.001$) compared to control (2.28). This was not uniform as several individuals presented with preserved RH-index indicating a preserved vascular and endothelial function. No significant changes were seen in RH-index through days 1-4 for CS patients. However, for postcardiotomy HF patients the RH-index increased through the observation period ($p = 0.019$) and was significantly higher at days 3 and 4 compared with baseline ($p = 0.011$). The longitudinal changes in RH-index were not associated with the concomitant changes in ADMA levels. We observed, however, a negative correlation between ADMA and RH-index ($r = -.633$, $p = 0.036$) at baseline for CS patients.

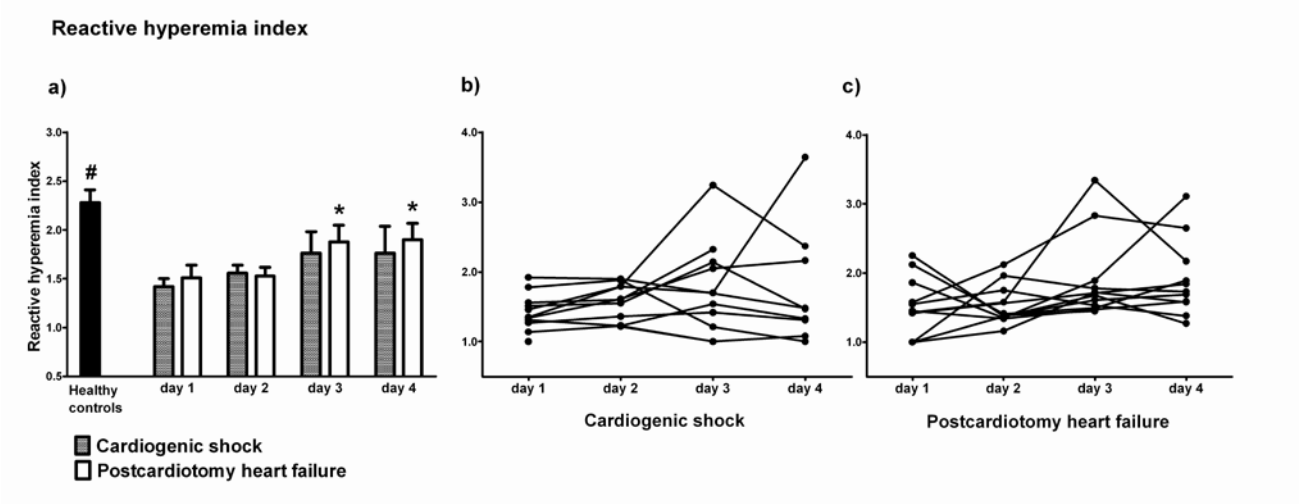

Figure 11. Reactive hyperemia index (RH-index) measured at days 1-4. a) Mean RH-index with SEM. Induvidual values of the repeated RH-index measurements for CS patients b) and postcardiotomy HF patients c). * $p < 0.05$ compared with day 1. # $p < 0.05$ compared with CS and postcardiotomy HF at day 1.

Interestingly, the resolving inflammatory response with falling levels of IL-6 and IL-8 observed in postcardiotomy HF patients was associated with the improving RH-index in these patients.

The measured ADMA levels are shown in figure 12. While baseline ADMA levels in CS and healthy controls were grossly similar (0.74 vs 0.79 µmol/L), they were on average significantly lower in postcardiotomy HF (0.55 µmol/L, $p = 0.04$). ADMA levels increased during the observation period for postcardiotomy HF patients, but remained unchanged in CS. There were no associations between day-to-day changes in ADMA and markers of inflammation and endothelial activation. However, patients who underwent cardiopulmonary resuscitation prior to CS onset had significantly higher baseline ADMA levels (1.05 vs 0.58 µmol/L, $p = 0.003$). Also, ADMA levels correlated with lactate levels measured at baseline ($r = .85$, $p = 0.001$) and the maximal lactate level measured within 48 hours ($r = .90$, $p < 0.001$).

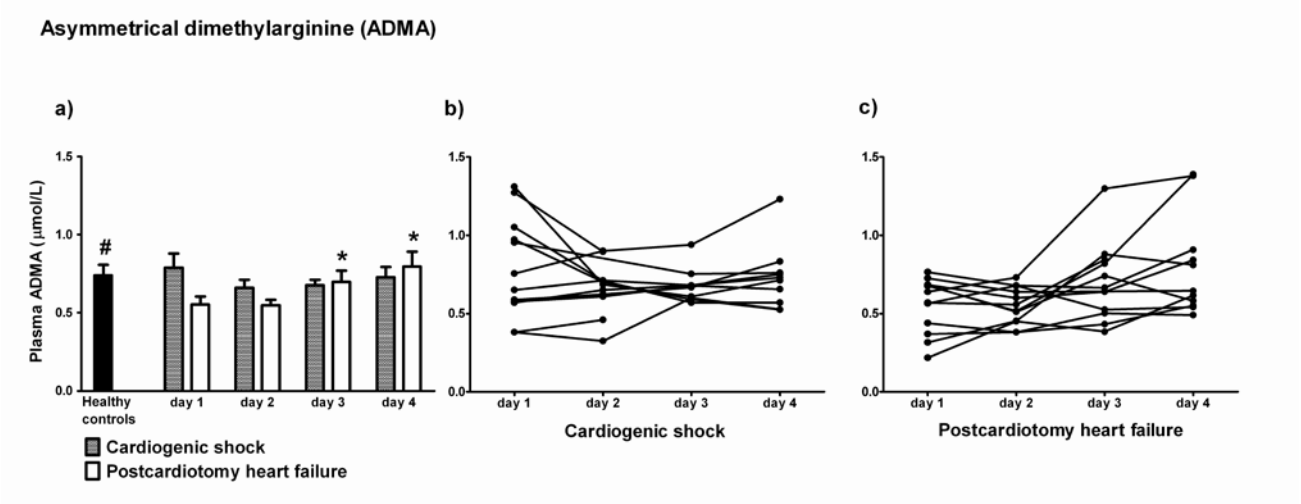

Figure 12. The course of plasma ADMA levels measured at days 1-4. a) Mean plasma ADMA with SEM. Individual changes in plasma ADMA levels for CS b) and postcardiotomy HF c). * $p < 0.05$ compared with day 1. $^{\#}$ $p < 0.05$ compared with postcardiotomy HF at day 1.

In order to look for relations between organ dysfunction and the measured variables we used the Sequential Organ Failure Assessment (SOFA) score[229] to assess the extent of organ dysfunction. CS patients with failure of an organ (excluding the central nervous system or cardiovascular system) had lower baseline RH –index (median 1.22 vs 1.51, $p = 0.01$) and elevated ADMA levels (median 0.58 vs. 1.05 µmol/L, $p = 0.005$). Moreover, SOFA score was correlated to baseline RH-index ($r = -.69$, $p = 0.014$), baseline ADMA levels ($r = .63$, $p = 0.028$) and levels of inflammatory markers IL-6 ($r = .62$, $p = 0.03$), IL-8 ($r = .78$, $p = 0.007$).

8.6 Discussion paper III and IV

Acute changes in endothelial function

The endothelial function following open heart surgery and at the onset of acute heart failure was impaired. In patients undergoing surgery, both elective patients and patients with subsequent postcardiotomy heart failure, this impairment was transient. A postoperative reduction in FMD has been described previously.[230, 231] This indicates that an acute systemic vascular dysfunction likely to influence on the vessels' ability to regulate vascular tone and blood flow takes place following open-heart surgery. Patients with cardiogenic shock showed a similar impairment in endothelial function compared with healthy controls. Prior studies have demonstrated attenuated increase in forearm blood flow during reactive hyperemia in patients with cardiogenic and septic shock.[86] A similar impairment has also been described in patients with severe sepsis and septic shock using peripheral arterial tonometry (similar to paper IV).[87, 88] The underlying mechanisms for this attenuation in endothelial functions are likely to be multifactorial, and probably involve a composite of prior vascular /endothelial function and a multitude of superimposed acute factors including vasoactive treatment. The peripheral vasculature in patients with severe hear failure, and in the course of acute heart failure, is subject to immense and changing stimuli from circulating hormones, the sympathetic nervous system and local metabolic factors capable of modulating both endothelial and vascular function.[232-234] The bioavailability of NO is considered the main determinant for endothelial function measured with both brachial ultrasound and PAT[81, 213, 217, 218] and thus these findings could indicate reduced NO bioavailability in these patients. We observed a negative correlation between the endogenous NO- inhibitor ADMA and endothelial function at baseline for CS patients that could suggest an inhibitory effect of ADMA on vascular function. There was, however, no apparent assosciation at baseline or

longitudinally during follow up between circulating levels of NO metabolites (open heart surgery patients) or ADMA (all patients). It is possible that attenuation of other important contributors to endothelium dependent dilation, such as prostacyclin or endothelium derived hyperpolarizing factor, play a bigger role in these patients. Also, fewer functional capillaries secondary to intravascular obstruction or oedema could blunt the reactive hyperemic response in critical disease.[235] Importantly, for patients undergoing surgery with CPB changes in blood viscosity due to perioperative blood loss and hemodilution could affect shear stress acting on the endothelium in the early postoperative period and the hemodilution will also affect the levels of circulating proteins and metabolites.[236]

Inflammation

In our study, patients with CS and postcardiotomy HF were subject to a profound inflammatory insult evident by excessive levels of the circulating inflammatory cytokines IL-6 and IL-8 that resolved during follow-up. This was markedly higher than reported in less severe heart failure and acute myocardial infarction[121, 122, 237] and comparable to the levels measured in sepsis[87, 120, 238]. High levels of IL-6 in CS patients were related to both low MAP and vasopressor requirements. The association between IL-6 levels and vasopressor support has been described earlier.[50] However, we observed no correlation at baseline or longitudinal association between the degree of inflammation and endothelial function to support that the observed vascular dysfunction was due to inflammation. Interestingly, the observed improvement in vasodilator function in postcardiotomy HF patients was associated with the resolving inflammatory response. Although the systemic inflammatory insult and endothelial activation inflicted by open-heart surgery and use of extra corporeal bypass is well described[239, 240], the cause and consequence of this on vascular distubances in CS is less well understood.

Asymmetrical Dimethylarginine

In line with previous observations, we observed suppressed postoperative levels of circulating ADMA after open-heart surgery.[241, 242] Initially low ADMA levels that increased during follow up were also observed at the onset of postcardiotomy HF. This could possibly be caused by a hemodiluting effect of extra corporeal circulation.[243, 244] In contrast to previous reports on acute decompensated heart failure and CS, ADMA levels in patients with CS were not different from healthy controls in our study.[146, 149]

ADMA levels did correlate with endothelial function at baseline for CS patients. This was not the case in postcardiotomy HF, and furthermore there were no associations between the longitudinal changes in ADMA and endothelial function in either group. Similarly, ADMA did not correlate with FMD measurements in patients undergoing open-heart surgery. Furthermore, ADMA did not relate to blood pressure or hemodynamic measurements. All together these studies gave no indication that the ADMA-DDAH pathway was responsible for the reduced endothelial function. The circulating plasma levels of ADMA do, however, not necessarily reflect intracellular ADMA levels or tissue levels in specific organs. Thus, a biological relevant NO-inhibitory effect can take place without a change in circulating levels.

In line with observations on patients with severe sepsis and critically ill patients, elevated ADMA levels were associated with the degree of organ dysfunction and elevated systemic lactate[151-153]. The observation that ADMA levels were highest in the patients that underwent CPR and had elevated lactate could indicate that ADMA reflects the degree of hypoperfusion during shock. Interestingly, patients with evidence of organ failure did not show any evidence of further accumulation of ADMA

8.7 Summary of results Paper V

The cultured late outgrowth endothelial progenitor cells expressed an endothelial phenotype as presented in fig 5. Moreover, EPCs stained positive for the CRLR and RAMP-2 and this was also found at the mRNA level using Real-time PCR (fig 5g). The addition of adrenomedullin (AM) increased proliferation in a dose-dependent manner. This was suppressed by the addition of the AM antagonist AM 22-52. Inhibition with the phosphatidylinositol 3-kinase (PI3K) inhibitor, wortmannin, abolished the AM-induced proliferation while no such effect was seen using a MEK ½ inhibitor. Also, the addition of AM produced a 36 % relative increase in tubular network formation in the EPC/fibroblast coculture assay, compared with controls (fig 12). A similar proangiogenic effect of AM stimulation was seen in the matrigel assay were we observed an 80 % increase in tubular network formation (fig 13). The proangiogenic and growth promoting effects of adrenomedullin was similar to that of VEGF. Addition of the AM antagonist AM 22-52 and PI3K inhibitor wortmannin blunted the increased tubule formation seen on the matrigel assay.

Non-stimulated EPCs secreted IL-6 and IL-8. There was an early augmentation in the secretion of these cytokines following AM stimulation (2 hrs) that was accompanied by a two-fold increase in the expression of IL-6 and IL-8 mRNA. There was, however, no change compared to non-stimulated cells at 20 hrs. Furthermore, AM did not seem to affect EPC release of VEGF or the VEGF and VEGF receptor 2 mRNA expression.

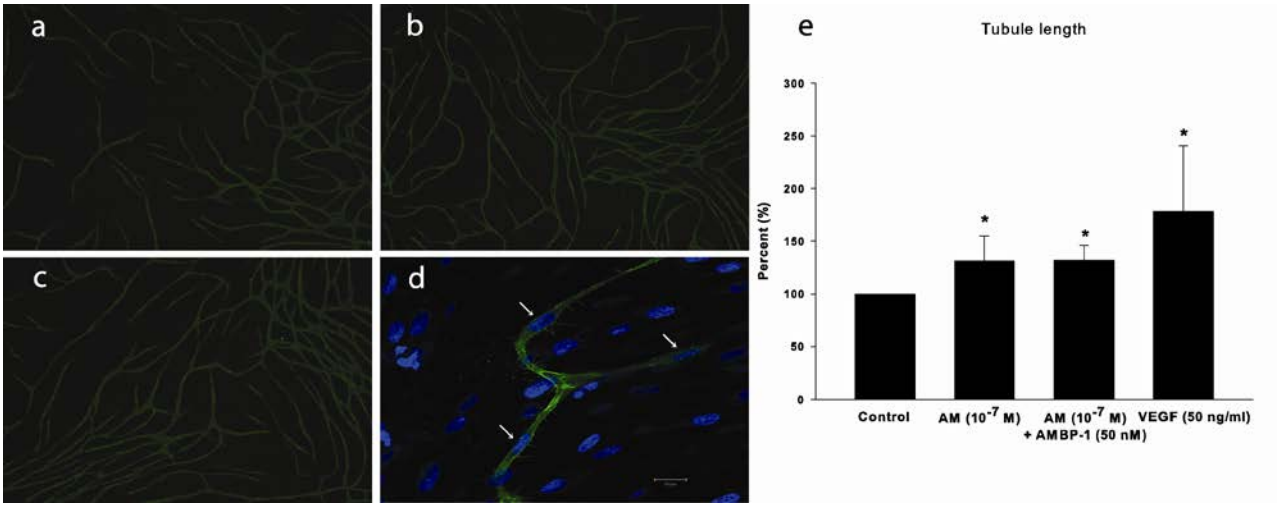

Figure 12. Tubule formation in EPC/fibroblast coculture. a-c) Representative images of CD31 stained tubules. a) Control. b) Addition of adrenomedullin (10^{-7} M). c) Addition of vascular endothelial growth factor (50 ng/ml). d) Confocal image of CD31 positive tubules. Cell nuclei counterstained with Draq5 (blue). e) Measured tubule length presented as percentage of control (±SD). * $p < 0.05$ compared with control. AMBP-1, adrenomedullin-binding protein 1.

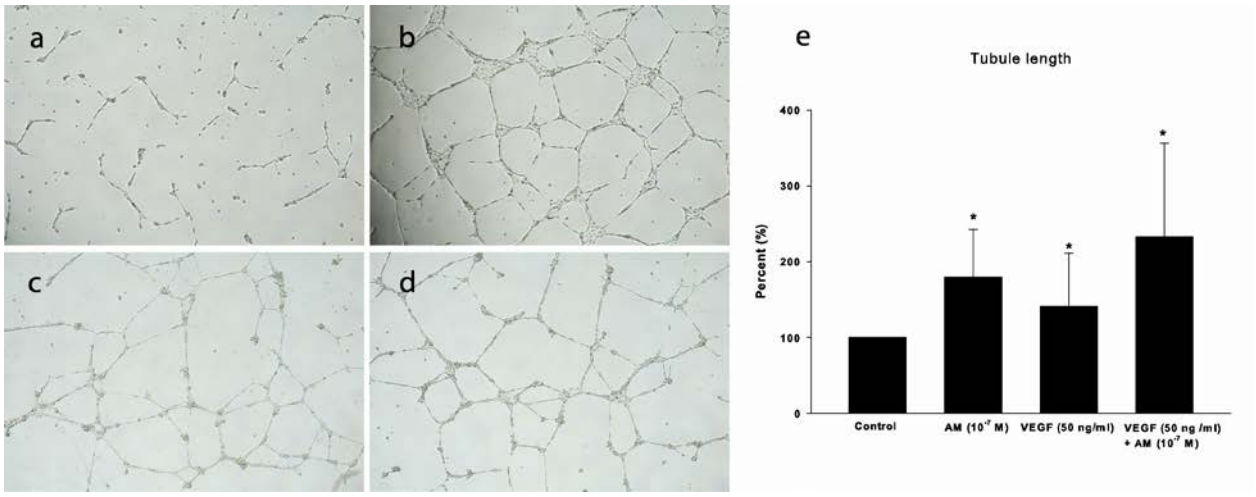

Figure 13. Tubule formation on matrigel matrix. a) Control. b) Addition of adrenomedullin (10^{-7} M). c) Addition of vascular endothelial growth factor (50 ng/ml). d) Addition of AM plus VEGF. e) Measured tubule length presented as percentage of control (±SD). * $p < 0.05$ compared with control.

8.8 Discussion paper V

This is the first study to demonstrate that human late outgrowth EPCs express the AM-1 (CRLR/RAMP-2) receptor complex, and importantly AM both enhances cell growth and the proangiogenic properties of these cells. Similar observations have previously been made for early outgrowth EPCs were AM suppress apoptosis and increase cell numbers.[207] Importantly, coadministration of AM augments the angiogenic effects of unspecified bone marrow-derived mononuclear cell transplantation in models of ischemia.[206, 209] These studies suggest a potent stimulatory role of AM in both EPCs and other cells with stem cell potential and thus its potential usefulness in therapeutic angiogenesis and vascular repair.

The AM-induced EPC proliferation and increased tubular network formation seem to be mediated through the PI3K/akt signalling pathway, demonstrated by the inhibitory effects of wortmannin. This pathway regulates several important steps in angiogenesis including endothelial cell proliferation, migration and capillary formation. This pathway also seems to mediate the antiapoptotic actions of AM on early EPCs.[207] While prior studies have shown that AM also induces proliferation of mature endothelial cells and angiogenesis through the activation of MAPK, this was not evident in the EPCs in our study using the MEK ½ inhibitor UO126.[204]

We found no evidence that AM acted through increased release of VEGF or expression of VEGF mRNA. Nor did AM affect the expression of the VEGFR -2. Prior reports on different populations of cultured endothelial cells have, however, shown conflicting results.[206, 210, 245-247] It has also been suggested that AM can act through transactivation of VEGFR-2 and thereby induce a proangiogenic signalling cascade.[246]

In line with earlier studies on late outgrowth EPCs, we found a secretion of IL-8 under basal culture conditions.[248, 249] They also secreted IL-6, as previously observed in mature endothelial cells.[250] Both IL-8 and IL-6 are shown to stimulate endothelial cell proliferation and act proangiogenic.[251-254] In EPCs, IL-8 seems to contribute substantially to the cells' paracrine mitogenic effects while IL-6 has been shown to increase cell proliferation and act proangiogenic.[248, 255] Our observations suggest that AM induces an early release of these cytokines. However, this stimulated release was small and transitory and a substantial proinflammatory effect was not observed. An indirect proangiogenic effect of AM through increased release of these cytokines cannot, however, not be ruled out.

9. Main conclusions

Paper I

- There was an extensive use of invasive revascularization in patients with cardiogenic shock following an acute coronary syndrome. Eligible patients who were not treated with early revascularization where in their eighties and presented with complicated comorbidities. The role of aggressive invasive therapy in this patient group is more illusive.
- Early mortality in CS was very high despite the extensive use of invasive revascularization and the majority of in-hospital deaths occurred within two days. More than one third of invasively treated patients died while in hospital adding further evidence to the limitations of invasive treatment in CS. There should be an effort to develop precise diagnostic algorithms to grade CS and its prognosis, to improve the initial medical therapy and probably to establish an early institution of temporary assist systems in selected patients.

- Despite extensive use of invasive revascularization in patients with less severe AHF compared to other reports, a significant number of patients did not receive this treatment. This could indicate a potential for increased use of early invasive treatment in this high risk population. The strikingly high intermediate-term mortality even compared to more severe forms of AHF, further underlines this.
- Mitral regurgitation was a contributing factor for the development of AHF in a significant number of patients. Mitral valve surgery was in our population only performed in the setting of acute papillary muscle rupture or endocarditis while mitral regurgitation secondary to left ventricular dysfunction was treated with revascularization and medical stabilization. To what extent mitral valve procedures should be done in the setting of AHF and cardiogenic shock, is for now unknown.
- Postcardiotomy patients constitute a significant proportion of the AHF population and should ideally be included in AHF registries. The sickest patients are often treated surgically, at times also with supportive invasive therapy for refractory circulatory shock. Also, postcardiotomy heart failure has many pathophysiological aspects in common with the other groups of AHF patients, and they need to be included in the development of new treatment algorithms.

Paper II

- Application of mechanical circulatory support by means of ventricular assist devices represents an important alternative for improving prognosis in severe acute heart failure (i.e. cardiogenic shock and postcardiotomy HF).
- In this retrospective study we found 12 % of these patients to be potential candidates for treatment with ventricular assist devices. The observed incidence in our material

indicates a mortality of potential VAD candidates with acute heart failure on 15/million inhabitants/year.

- Female patients with inadequate cardiac pump function after valve replacement were at the highest risk of dying despite maximal conventional treatment efforts.

Paper III

- Open heart surgery with the use of cardiopulmonary bypass induces a temporary attenuation in flow mediated dilation in both patients with healthy endothelium and manifest atherosclerotic disease.
- There were no association between circulating levels of NOx (nitrite + nitrate), ADMA (asymmetric dimethylarginine) and the attenuated endothelial response.

Paper IV

- Cardiogenic shock and postcardiotomy HF were characterized by initially elevated levels of inflammatory cytokines suggestive of a profound inflammatory insult.
- This acute inflammatory response was accompanied by an attenuated vasodilator function in both groups. Vasodilator function improved during the observation period for patients with postcardiotomy HF and this improvement was associated with a resolving inflammatory response. This relation was, however, not evident in CS patients. The repeated assessment of vasodilator function revealed a substantial individual day to day variation suggesting that vascular function in acute disease is not static.
- The circulating levels of the endogenous NO-inhibitor ADMA did not relate to the degree of inflammation or the day-to-day changes in vasodilator function.

- In CS, ADMA, vasodilator function and levels of inflammatory cytokines correlated to the overall degree of organ dysfunction and to hepatic dysfunction in particular.
- The increased ADMA levels observed in patients who underwent CPR and/or had elevated arterial lactate levels suggest a relation between ADMA and the degree of hypoperfusion in CS.
- This study demonstrated that EndoPAT was a feasible technique for repeated measures of peripheral vasodilator function in the intensive care setting.

Paper V

- Late outgrowth endothelial progenitor cells possess an endothelial phenotype and express the adrenomedullin 1 receptor complex (CRLR/RAMP-2).
- Adrenomedullin stimulation induced proliferation of EPCs and a marked increase in formation of tubular networks in vitro in a similar fashion to that of VEGF.
- The proliferative and proangiogenetic effects of adrenomedullin seemed to be mediated through the PI3K/Akt signalling pathway.
- Adrenomedullin did not seem to influence the paracrine properties of EPCs, i.e release of proinflammatory and proangiogenic cytokines and chemokines.

10. References

1 Hermansen SE, Hansen M, Roaldsen M, Muller S, How OJ, Myrmel T. Utilization and outcome of coronary revascularization and valve procedures in acute heart failure--an evaluation based on the classification from the European Society of Cardiology. *Interact Cardiovasc Thorac Surg* 2008 October;7(5):833-8.

2 Hermansen SE, Hansen M, Roaldsen M, Muller S, How OJ, Myrmel T. How many acute heart failure patients need a ventricular assist device? *Scand Cardiovasc J* 2008 April;42(2):118-24.

3 Hermansen SE, Kalstad T, How OJ, Myrmel T. Inflammation and reduced endothelial function in the course of severe acute heart failure. *Transl Res* 2011 March;157(3):117-27.

4 Hermansen SE, Lund T, Kalstad T, Ytrehus K, Myrmel T. Adrenomedullin augments the angiogenic potential of late outgrowth endothelial progenitor cells. *Am J Physiol Cell Physiol* 2011 April;300(4):C783-C791.

5 Hermansen SE, Naesheim T, How OJ, Myrmel T. Circulatory assistance in acute heart failure--where do we go from here? *Scand Cardiovasc J* 2009 August;43(4):211-6.

6 Nieminen MS, Bohm M, Cowie MR et al. Executive summary of the guidelines on the diagnosis and treatment of acute heart failure: the Task Force on Acute Heart Failure of the European Society of Cardiology. *Eur Heart J* 2005 February;26(4):384-416.

7 Rudiger A, Harjola VP, Muller A et al. Acute heart failure: clinical presentation, one-year mortality and prognostic factors. *Eur J Heart Fail* 2005 June;7(4):662-70.

8 Follath F, Yilmaz MB, Delgado JF et al. Clinical presentation, management and outcomes in the Acute Heart Failure Global Survey of Standard Treatment (ALARM-HF). *Intensive Care Med* 2011 April;37(4):619-26.

9 Tavazzi L, Maggioni AP, Lucci D et al. Nationwide survey on acute heart failure in cardiology ward services in Italy. *Eur Heart J* 2006 May;27(10):1207-15.

10 Adams KF, Jr., Fonarow GC, Emerman CL et al. Characteristics and outcomes of patients hospitalized for heart failure in the United States: rationale, design, and preliminary observations from the first 100,000 cases in the Acute Decompensated Heart Failure National Registry (ADHERE). *Am Heart J* 2005 February;149(2):209-16.

11 Zannad F, Mebazaa A, Juilliere Y et al. Clinical profile, contemporary management and one-year mortality in patients with severe acute heart failure syndromes: The EFICA study. *Eur J Heart Fail* 2006 March 2.

12 Nieminen MS, Brutsaert D, Dickstein K et al. EuroHeart Failure Survey II (EHFS II): a survey on hospitalized acute heart failure patients: description of population. *Eur Heart J* 2006 September 25.

13 Harjola VP, Follath F, Nieminen MS et al. Characteristics, outcomes, and predictors of mortality at 3 months and 1 year in patients hospitalized for acute heart failure. *Eur J Heart Fail* 2010 March;12(3):239-48.

14 Jeger RV, Radovanovic D, Hunziker PR et al. Ten-year trends in the incidence and treatment of cardiogenic shock. *Ann Intern Med* 2008 November 4;149(9):618-26.

15 Zeymer U, Vogt A, Zahn R et al. Predictors of in-hospital mortality in 1333 patients with acute myocardial infarction complicated by cardiogenic shock treated with primary percutaneous coronary intervention (PCI); Results of the primary PCI registry of the Arbeitsgemeinschaft Leitende Kardiologische Krankenhausarzte (ALKK). *Eur Heart J* 2004 February;25(4):322-8.

16 Babaev A, Frederick PD, Pasta DJ, Every N, Sichrovsky T, Hochman JS. Trends in management and outcomes of patients with acute myocardial infarction complicated by cardiogenic shock. *JAMA* 2005 July 27;294(4):448-54.

17 Jeger RV, Assmann SF, Yehudai L, Ramanathan K, Farkouh ME, Hochman JS. Causes of death and re-hospitalization in cardiogenic shock. *Acute Card Care* 2007;9(1):25-33.

18 Hasdai D, Behar S, Wallentin L et al. A prospective survey of the characteristics, treatments and outcomes of patients with acute coronary syndromes in Europe and the Mediterranean basin; the Euro Heart Survey of Acute Coronary Syndromes (Euro Heart Survey ACS). *Eur Heart J* 2002 August;23(15):1190-201.

19 Fox KA, Steg PG, Eagle KA et al. Decline in rates of death and heart failure in acute coronary syndromes, 1999-2006. *JAMA* 2007 May 2;297(17):1892-900.

20 Hasdai D, Behar S, Wallentin L et al. A prospective survey of the characteristics, treatments and outcomes of patients with acute coronary syndromes in Europe and the Mediterranean basin; the Euro Heart Survey of Acute Coronary Syndromes (Euro Heart Survey ACS). *Eur Heart J* 2002 August;23(15):1190-201.

21 Fang J, Mensah GA, Alderman MH, Croft JB. Trends in acute myocardial infarction complicated by cardiogenic shock, 1979-2003, United States. *Am Heart J* 2006 December;152(6):1035-41.

22 Sleeper LA, Ramanathan K, Picard MH et al. Functional status and quality of life after emergency revascularization for cardiogenic shock complicating acute myocardial infarction. *J Am Coll Cardiol* 2005 July 19;46(2):266-73.

23 Singh M, White J, Hasdai D et al. Long-term outcome and its predictors among patients with ST-segment elevation myocardial infarction complicated by shock: insights from the GUSTO-I trial. *J Am Coll Cardiol* 2007 October 30;50(18):1752-8.

24 Rao V, Ivanov J, Weisel RD, Ikonomidis JS, Christakis GT, David TE. Predictors of low cardiac output syndrome after coronary artery bypass. *J Thorac Cardiovasc Surg* 1996 July;112(1):38-51.

25 Hausmann H, Potapov EV, Koster A et al. Prognosis after the implantation of an intra-aortic balloon pump in cardiac surgery calculated with a new score. *Circulation* 2002 September 24;106(12 Suppl 1):I203-I206.

26 Pennington DG, Swartz M, Codd JE, Merjavy JP, Kaiser GC. Intraaortic balloon pumping in cardiac surgical patients: a nine-year experience. *Ann Thorac Surg* 1983 August;36(2):125-31.

27 Samuels LE, Kaufman MS, Thomas MP, Holmes EC, Brockman SK, Wechsler AS. Pharmacological criteria for ventricular assist device insertion following postcardiotomy shock: experience with the Abiomed BVS system. *J Card Surg* 1999 July;14(4):288-93.

28 Nasir A, Bonde P, Graham AN. Ventricular assist device therapy in post-cardiotomy cardiogenic shock: historical outcomes and current trends. *Interact Cardiovasc Thorac Surg* 2012 February 7.

29 Doll N, Kiaii B, Borger M et al. Five-year results of 219 consecutive patients treated with extracorporeal membrane oxygenation for refractory postoperative cardiogenic shock. *Ann Thorac Surg* 2004 January;77(1):151-7.

30 Hernandez AF, Grab JD, Gammie JS et al. A decade of short-term outcomes in post cardiac surgery ventricular assist device implantation: data from the Society of Thoracic Surgeons' National Cardiac Database. *Circulation* 2007 August 7;116(6):606-12.

31 Helman DN, Morales DL, Edwards NM et al. Left ventricular assist device bridge-to-transplant network improves survival after failed cardiotomy. *Ann Thorac Surg* 1999 October;68(4):1187-94.

32 Paul S, Leacche M, Unic D et al. Determinants of outcomes for postcardiotomy VAD placement: an 11-year, two-institution study. *J Card Surg* 2006 May;21(3):234-7.

33 Pae WE, Jr., Miller CA, Matthews Y, Pierce WS. Ventricular assist devices for postcardiotomy cardiogenic shock. A combined registry experience. *J Thorac Cardiovasc Surg* 1992 September;104(3):541-52.

34 Hochman JS, Sleeper LA, Webb JG et al. Early revascularization in acute myocardial infarction complicated by cardiogenic shock. SHOCK Investigators. Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock. *N Engl J Med* 1999 August 26;341(9):625-34.

35 Davila-Roman VG, Waggoner AD, Hopkins WE, Barzilai B. Right ventricular dysfunction in low output syndrome after cardiac operations: assessment by transesophageal echocardiography. *Ann Thorac Surg* 1995 October;60(4):1081-6.

36 Argenziano M, Chen JM, Choudhri AF et al. Management of vasodilatory shock after cardiac surgery: identification of predisposing factors and use of a novel pressor agent. *J Thorac Cardiovasc Surg* 1998 December;116(6):973-80.

37 Fincke R, Hochman JS, Lowe AM et al. Cardiac power is the strongest hemodynamic correlate of mortality in cardiogenic shock: a report from the SHOCK trial registry. *J Am Coll Cardiol* 2004 July 21;44(2):340-8.

38 Jeger RV, Lowe AM, Buller CE et al. Hemodynamic Parameters Are Prognostically Important in Cardiogenic Shock But Similar Following Early Revascularization or Initial Medical Stabilization: A Report From the SHOCK Trial. *Chest* 2007 December;132(6):1794-803.

39 Picard MH, Davidoff R, Sleeper LA et al. Echocardiographic predictors of survival and response to early revascularization in cardiogenic shock. *Circulation* 2003 January 21;107(2):279-84.

40 Valente S, Lazzeri C, Vecchio S et al. Predictors of in-hospital mortality after percutaneous coronary intervention for cardiogenic shock. *Int J Cardiol* 2007 January 8;114(2):176-82.

41 Hochman JS, Buller CE, Sleeper LA et al. Cardiogenic shock complicating acute myocardial infarction--etiologies, management and outcome: a report from the SHOCK Trial Registry. SHould we emergently revascularize Occluded Coronaries for cardiogenic shocK? *J Am Coll Cardiol* 2000 September;36(3 Suppl A):1063-70.

42 Hollenberg SM, Kavinsky CJ, Parrillo JE. Cardiogenic shock. *Ann Intern Med* 1999 July 6;131(1):47-59.

43 Page DL, Caulfield JB, Kastor JA, DeSanctis RW, Sanders CA. Myocardial changes associated with cardiogenic shock. *N Engl J Med* 1971 July 15;285(3):133-7.

44 Alonso DR, Scheidt S, Post M, Killip T. Pathophysiology of cardiogenic shock. Quantification of myocardial necrosis, clinical, pathologic and electrocardiographic correlations. *Circulation* 1973 September;48(3):588-96.

45 Hochman JS. Cardiogenic shock complicating acute myocardial infarction: expanding the paradigm. *Circulation* 2003 June 24;107(24):2998-3002.

46 Kohsaka S, Menon V, Lowe AM et al. Systemic inflammatory response syndrome after acute myocardial infarction complicated by cardiogenic shock. *Arch Intern Med* 2005 July 25;165(14):1643-50.

47 Menon V, Slater JN, White HD, Sleeper LA, Cocke T, Hochman JS. Acute myocardial infarction complicated by systemic hypoperfusion without hypotension: report of the SHOCK trial registry. *Am J Med* 2000 April 1;108(5):374-80.

48 Sheu JJ, Tsai TH, Lee FY et al. Early extracorporeal membrane oxygenator-assisted primary percutaneous coronary intervention improved 30-day clinical outcomes in patients with ST-segment elevation myocardial infarction complicated with profound cardiogenic shock. *Crit Care Med* 2010 September;38(9):1810-7.

49 Tarantini G, Ramondo A, Isabella G et al. Pressure response to vasopressors and mortality after direct angioplasty for cardiogenic shock. *Int J Cardiol* 2004 April;94(2-3):197-202.

50 Geppert A, Dorninger A, Delle-Karth G, Zorn G, Heinz G, Huber K. Plasma concentrations of interleukin-6, organ failure, vasopressor support, and successful coronary revascularization in predicting 30-day mortality of patients with cardiogenic shock complicating acute myocardial infarction. *Crit Care Med* 2006 August;34(8):2035-42.

51 Katz JN, Stebbins AL, Alexander JH et al. Predictors of 30-day mortality in patients with refractory cardiogenic shock following acute myocardial infarction despite a patent infarct artery. *Am Heart J* 2009 October;158(4):680-7.

52 Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. *N Engl J Med* 2001 August 23;345(8):588-95.

53 Van de Werf F, Bax J, Betriu A et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J* 2008 December;29(23):2909-45.

54 Hamm CW, Bassand JP, Agewall S et al. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2011 December;32(23):2999-3054.

55 Wijns W, Kolh P, Danchin N et al. Guidelines on myocardial revascularization. *Eur Heart J* 2010 October;31(20):2501-55.

56 Dauerman HL, Goldberg RJ, White K et al. Revascularization, stenting, and outcomes of patients with acute myocardial infarction complicated by cardiogenic shock. *Am J Cardiol* 2002 October 15;90(8):838-42.

57 Iakobishvili Z, Behar S, Boyko V, Battler A, Hasdai D. Does current treatment of cardiogenic shock complicating the acute coronary syndromes comply with guidelines? *Am Heart J* 2005 January;149(1):98-103.

58 Dzavik V, Sleeper LA, Cocke TP et al. Early revascularization is associated with improved survival in elderly patients with acute myocardial infarction complicated by cardiogenic shock: a report from the SHOCK Trial Registry. *Eur Heart J* 2003 May;24(9):828-37.

59 Antman EM, Anbe DT, Armstrong PW et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction; A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of patients with acute myocardial infarction). *J Am Coll Cardiol* 2004 August 4;44(3):E1-E211.

60 Jacobs AK, French JK, Col J et al. Cardiogenic shock with non-ST-segment elevation myocardial infarction: a report from the SHOCK Trial Registry. SHould we emergently revascularize Occluded coronaries for Cardiogenic shocK? *J Am Coll Cardiol* 2000 September;36(3 Suppl A):1091-6.

61 Eagle KA, Guyton RA, Davidoff R et al. ACC/AHA 2004 guideline update for coronary artery bypass graft surgery: summary article: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1999 Guidelines for Coronary Artery Bypass Graft Surgery). *Circulation* 2004 August 31;110(9):1168-76.

62 White HD, Assmann SF, Sanborn TA et al. Comparison of percutaneous coronary intervention and coronary artery bypass grafting after acute myocardial infarction complicated by cardiogenic shock: results from the Should We Emergently Revascularize Occluded Coronaries for Cardiogenic Shock (SHOCK) trial. *Circulation* 2005 September 27;112(13):1992-2001.

63 Morrison DA, Sethi G, Sacks J et al. Percutaneous coronary intervention versus coronary artery bypass graft surgery for patients with medically refractory myocardial ischemia and risk factors for adverse outcomes with bypass: a multicenter, randomized trial. Investigators of the Department of Veterans Affairs Cooperative Study #385, the Angina With Extremely Serious Operative Mortality Evaluation (AWESOME). *J Am Coll Cardiol* 2001 July;38(1):143-9.

64 van der Schaaf RJ, Vis MM, Sjauw KD et al. Impact of multivessel coronary disease on long-term mortality in patients with ST-elevation myocardial infarction is due to the presence of a chronic total occlusion. *Am J Cardiol* 2006 November 1;98(9):1165-9.

65 van der Schaaf RJ, Timmer JR, Ottervanger JP et al. Long-term impact of multivessel disease on cause-specific mortality after ST elevation myocardial infarction treated with reperfusion therapy. *Heart* 2006 December;92(12):1760-3.

66 Spencer FA, Meyer TE, Gore JM, Goldberg RJ. Heterogeneity in the management and outcomes of patients with acute myocardial infarction complicated by heart failure: the National Registry of Myocardial Infarction. *Circulation* 2002 June 4;105(22):2605-10.

67 Steg PG, Dabbous OH, Feldman LJ et al. Determinants and prognostic impact of heart failure complicating acute coronary syndromes: observations from the Global Registry of Acute Coronary Events (GRACE). *Circulation* 2004 February 3;109(4):494-9.

68 Thompson CR, Buller CE, Sleeper LA et al. Cardiogenic shock due to acute severe mitral regurgitation complicating acute myocardial infarction: a report from the SHOCK Trial Registry. SHould we use emergently revascularize Occluded Coronaries in cardiogenic shocK? *J Am Coll Cardiol* 2000 September;36(3 Suppl A):1104-9.

69 Leshnower BG, Gleason TG, O'Hara ML et al. Safety and efficacy of left ventricular assist device support in postmyocardial infarction cardiogenic shock. *Ann Thorac Surg* 2006 April;81(4):1365-70.

70 Kar B, Gregoric ID, Basra SS, Idelchik GM, Loyalka P. The percutaneous ventricular assist device in severe refractory cardiogenic shock. *J Am Coll Cardiol* 2011 February 8;57(6):688-96.

71 Vanzetto G, Akret C, Bach V et al. [Percutaneous extracorporeal life support in acute severe hemodynamic collapses: single centre experience in 100 consecutive patients]. *Can J Cardiol* 2009 June;25(6):e179-e186.

72 Nichol G, Karmy-Jones R, Salerno C, Cantore L, Becker L. Systematic review of percutaneous cardiopulmonary bypass for cardiac arrest or cardiogenic shock states. *Resuscitation* 2006 September;70(3):381-94.

73 Chen JS, Ko WJ, Yu HY et al. Analysis of the outcome for patients experiencing myocardial infarction and cardiopulmonary resuscitation refractory to conventional therapies necessitating extracorporeal life support rescue. *Crit Care Med* 2006 April;34(4):950-7.

74 Liden H, Wiklund L, Haraldsson A, Berglin E, Hultman J, Dellgren G. Temporary circulatory support with extra corporeal membrane oxygenation in adults with refractory cardiogenic shock. *Scand Cardiovasc J* 2009 August;43(4):226-32.

75 Tayara W, Starling RC, Yamani MH, Wazni O, Jubran F, Smedira N. Improved survival after acute myocardial infarction complicated by cardiogenic shock with circulatory support and transplantation: comparing aggressive intervention with conservative treatment. *J Heart Lung Transplant* 2006 May;25(5):504-9.

76 Entwistle JW, III, Bolno PB, Holmes E, Samuels LE. Improved survival with ventricular assist device support in cardiogenic shock after myocardial infarction. *Heart Surg Forum* 2003;6(5):316-9.

77 Chen JM, DeRose JJ, Slater JP et al. Improved survival rates support left ventricular assist device implantation early after myocardial infarction. *J Am Coll Cardiol* 1999 June;33(7):1903-8.

78 Dang NC, Topkara VK, Leacche M, John R, Byrne JG, Naka Y. Left ventricular assist device implantation after acute anterior wall myocardial infarction and cardiogenic shock: a two-center study. *J Thorac Cardiovasc Surg* 2005 September;130(3):693-8.

79 Chen YS, Lin JW, Yu HY et al. Cardiopulmonary resuscitation with assisted extracorporeal life-support versus conventional cardiopulmonary resuscitation in adults with in-hospital cardiac arrest: an observational study and propensity analysis. *Lancet* 2008 August 16;372(9638):554-61.

80 Vane JR, Anggard EE, Botting RM. Regulatory functions of the vascular endothelium. *N Engl J Med* 1990 July 5;323(1):27-36.

81 Deanfield J, Donald A, Ferri C et al. Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005 January;23(1):7-17.

82 Widlansky ME, Gokce N, Keaney JF, Jr., Vita JA. The clinical implications of endothelial dysfunction. *J Am Coll Cardiol* 2003 October 1;42(7):1149-60.

83 Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 2002 April 2;105(13):1567-72.

84 Fichtlscherer S, Breuer S, Zeiher AM. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. *Circulation* 2004 October 5;110(14):1926-32.

85 Vallet B. Bench-to-bedside review: endothelial cell dysfunction in severe sepsis: a role in organ dysfunction? *Crit Care* 2003 April;7(2):130-8.

86 Kirschenbaum LA, Astiz ME, Rackow EC, Saha DC, Lin R. Microvascular response in patients with cardiogenic shock. *Crit Care Med* 2000 May;28(5):1290-4.

87 Davis JS, Yeo TW, Thomas JH et al. Sepsis-associated microvascular dysfunction measured by peripheral arterial tonometry: an observational study. *Crit Care* 2009;13(5):R155.

88 Davis JS, Darcy CJ, Yeo TW et al. Asymmetric dimethylarginine, endothelial nitric oxide bioavailability and mortality in sepsis. *PLoS One* 2011;6(2):e17260.

89 Agnoletti L, Curello S, Bachetti T et al. Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor-alpha. *Circulation* 1999 November 9;100(19):1983-91.

90 Colombo PC, Banchs JE, Celaj S et al. Endothelial cell activation in patients with decompensated heart failure. *Circulation* 2005 January 4;111(1):58-62.

91 Clapp BR, Hingorani AD, Kharbanda RK et al. Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. *Cardiovasc Res* 2004 October 1;64(1):172-8.

92 Wang P, Ba ZF, Chaudry IH. Administration of tumor necrosis factor-alpha in vivo depresses endothelium-dependent relaxation. *Am J Physiol* 1994 June;266(6 Pt 2):H2535-H2541.

93 Engelberger RP, Pittet YK, Henry H et al. Acute endotoxemia inhibits microvascular nitric oxide-dependent vasodilation in humans. *Shock* 2011 January;35(1):28-34.

94 Draisma A, Bemelmans R, van der Hoeven JG, Spronk P, Pickkers P. Microcirculation and vascular reactivity during endotoxemia and endotoxin tolerance in humans. *Shock* 2009 June;31(6):581-5.

95 Bhagat K, Vallance P. Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. *Circulation* 1997 November 4;96(9):3042-7.

96 Hingorani AD, Cross J, Kharbanda RK et al. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. *Circulation* 2000 August 29;102(9):994-9.

97 Bhagat K, Moss R, Collier J, Vallance P. Endothelial "stunning" following a brief exposure to endotoxin: a mechanism to link infection and infarction? *Cardiovasc Res* 1996 November;32(5):822-9.

98 Zhang C, Xu X, Potter BJ et al. TNF-alpha contributes to endothelial dysfunction in ischemia/reperfusion injury. *Arterioscler Thromb Vasc Biol* 2006 March;26(3):475-80.

99 Iversen PO, Nicolaysen A, Kvernebo K, Benestad HB, Nicolaysen G. Human cytokines modulate arterial vascular tone via endothelial receptors. *Pflugers Arch* 1999 December;439(1-2):93-100.

100 Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJ. Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. *Am J Cardiol* 1997 May 15;79(10):1426-30.

101 Sato Y, Takatsu Y, Kataoka K et al. Serial circulating concentrations of C-reactive protein, interleukin (IL)-4, and IL-6 in patients with acute left heart decompensation. *Clin Cardiol* 1999 December;22(12):811-3.

102 Peschel T, Schonauer M, Thiele H, Anker SD, Schuler G, Niebauer J. Invasive assessment of bacterial endotoxin and inflammatory cytokines in patients with acute heart failure. *Eur J Heart Fail* 2003 October;5(5):609-14.

103 Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 1990 July 26;323(4):236-41.

104 Anker SD, Volterrani M, Egerer KR et al. Tumour necrosis factor alpha as a predictor of impaired peak leg blood flow in patients with chronic heart failure. *QJM* 1998 March;91(3):199-203.

105 Katz SD, Rao R, Berman JW et al. Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure. Relation to nitric oxide-dependent vasodilation in the forearm circulation. *Circulation* 1994 July;90(1):12-6.

106 Vanderheyden M, Kersschot E, Paulus WJ. Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm. Serial assessment in patients with congestive heart failure. *Eur Heart J* 1998 May;19(5):747-52.

107 Smith CJ, Sun D, Hoegler C et al. Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. *Circ Res* 1996 January;78(1):58-64.

108 Fichtlscherer S, Rossig L, Breuer S, Vasa M, Dimmeler S, Zeiher AM. Tumor necrosis factor antagonism with etanercept improves systemic endothelial vasoreactivity in patients with advanced heart failure. *Circulation* 2001 December 18;104(25):3023-5.

109 Mann DL, McMurray JJ, Packer M et al. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation* 2004 April 6;109(13):1594-602.

110 Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res* 2002 November 29;91(11):988-98.

111 Anker SD, Coats AJ. How to RECOVER from RENAISSANCE? The significance of the results of RECOVER, RENAISSANCE, RENEWAL and ATTACH. *Int J Cardiol* 2002 December;86(2-3):123-30.

112 Testa L, Van Gaal WJ, Bhindi R et al. Pexelizumab in ischemic heart disease: a systematic review and meta-analysis on 15,196 patients. *J Thorac Cardiovasc Surg* 2008 October;136(4):884-93.

113 Eikelboom JW, O'Donnell M. Pexelizumab does not "complement" percutaneous coronary intervention in patients with ST-elevation myocardial infarction. *JAMA* 2007 January 3;297(1):91-2.

114 Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT. Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. *Circulation* 2003 July 1;107(25):3133-40.

115 Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. *N Engl J Med* 2004 December 16;351(25):2611-8.

116 Tonetti MS, D'Aiuto F, Nibali L et al. Treatment of periodontitis and endothelial function. *N Engl J Med* 2007 March 1;356(9):911-20.

117 Vallance P, Collier J, Bhagat K. Infection, inflammation, and infarction: does acute endothelial dysfunction provide a link? *Lancet* 1997 May 10;349(9062):1391-2.

118 Neumann FJ, Ott I, Gawaz M et al. Cardiac release of cytokines and inflammatory responses in acute myocardial infarction. *Circulation* 1995 August 15;92(4):748-55.

119 Guillen I, Blanes M, Gomez-Lechon MJ, Castell JV. Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6. *Am J Physiol* 1995 August;269(2 Pt 2):R229-R235.

120 Geppert A, Steiner A, Zorn G et al. Multiple organ failure in patients with cardiogenic shock is associated with high plasma levels of interleukin-6. *Crit Care Med* 2002 September;30(9):1987-94.

121 Theroux P, Armstrong PW, Mahaffey KW et al. Prognostic significance of blood markers of inflammation in patients with ST-segment elevation myocardial infarction undergoing primary angioplasty and effects of pexelizumab, a C5 inhibitor: a substudy of the COMMA trial. *Eur Heart J* 2005 October;26(19):1964-70.

122 Debrunner M, Schuiki E, Minder E et al. Proinflammatory cytokines in acute myocardial infarction with and without cardiogenic shock. *Clin Res Cardiol* 2008 May;97(5):298-305.

123 Fan ZX, Hua Q, Li YP, Liu RK, Yang Z. Interleukin-6, but Not Soluble Adhesion Molecules, Predicts a Subsequent Mortality from Cardiovascular Disease in Patients with Acute ST-Segment Elevation Myocardial Infarction. *Cell Biochem Biophys* 2011 November;61(2):443-8.

124 Li H, Forstermann U. Nitric oxide in the pathogenesis of vascular disease. *J Pathol* 2000 February;190(3):244-54.

125 Shah AM. Inducible nitric oxide synthase and cardiovascular disease. *Cardiovasc Res* 2000 January 1;45(1):148-55.

126 Ballinger SW, Patterson C, Yan CN et al. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. *Circ Res* 2000 May 12;86(9):960-6.

127 Alexander JH, Reynolds HR, Stebbins AL et al. Effect of tilarginine acetate in patients with acute myocardial infarction and cardiogenic shock: the TRIUMPH randomized controlled trial. *JAMA* 2007 April 18;297(15):1657-66.

128 Cauwels A. Nitric oxide in shock. *Kidney Int* 2007 September;72(5):557-65.

129 Lopez A, Lorente JA, Steingrub J et al. Multiple-center, randomized, placebo-controlled, double-blind study of the nitric oxide synthase inhibitor 546C88: effect on survival in patients with septic shock. *Crit Care Med* 2004 January;32(1):21-30.

130 Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992 March 7;339(8793):572-5.

131 Boger RH. Asymmetric dimethylarginine (ADMA): a novel risk marker in cardiovascular medicine and beyond. *Ann Med* 2006;38(2):126-36.

132 Valkonen VP, Paiva H, Salonen JT et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet* 2001 December 22;358(9299):2127-8.

133 Lu TM, Ding YA, Lin SJ, Lee WS, Tai HC. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. *Eur Heart J* 2003 November;24(21):1912-9.

134 Krempl TK, Maas R, Sydow K, Meinertz T, Boger RH, Kahler J. Elevation of asymmetric dimethylarginine in patients with unstable angina and recurrent cardiovascular events. *Eur Heart J* 2005 September;26(18):1846-51.

135 Stuhlinger MC, Oka RK, Graf EE et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. *Circulation* 2003 August 26;108(8):933-8.

136 Ohnishi M, Wada A, Tsutamoto T et al. Endothelin stimulates an endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine, in experimental heart failure. *Clin Sci (Lond)* 2002 August;103 Suppl 48:241S-4S.

137 Leiper J, Nandi M, Torondel B et al. Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat Med* 2007 February;13(2):198-203.

138 McBride AE, Silver PA. State of the arg: protein methylation at arginine comes of age. *Cell* 2001 July 13;106(1):5-8.

139 Teerlink T. ADMA metabolism and clearance. *Vasc Med* 2005 July;10 Suppl 1:S73-S81.

140 Teerlink T, Luo Z, Palm F, Wilcox CS. Cellular ADMA: regulation and action. *Pharmacol Res* 2009 December;60(6):448-60.

141 Achan V, Broadhead M, Malaki M et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arterioscler Thromb Vasc Biol* 2003 August 1;23(8):1455-9.

142 Leiper J, Murray-Rust J, McDonald N, Vallance P. S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase. *Proc Natl Acad Sci U S A* 2002 October 15;99(21):13527-32.

143 Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 1999 June 22;99(24):3092-5.

144 Ueda S, Kato S, Matsuoka H et al. Regulation of cytokine-induced nitric oxide synthesis by asymmetric dimethylarginine: role of dimethylarginine dimethylaminohydrolase. *Circ Res* 2003 February 7;92(2):226-33.

145 Zoccali C, Maas R, Cutrupi S et al. Asymmetric dimethyl-arginine (ADMA) response to inflammation in acute infections. *Nephrol Dial Transplant* 2007 March;22(3):801-6.

146 Nicholls SJ, Wang Z, Koeth R et al. Metabolic profiling of arginine and nitric oxide pathways predicts hemodynamic abnormalities and mortality in patients with cardiogenic shock after acute myocardial infarction. *Circulation* 2007 November 13;116(20):2315-24.

147 Feng Q, Lu X, Fortin AJ et al. Elevation of an endogenous inhibitor of nitric oxide synthesis in experimental congestive heart failure. *Cardiovasc Res* 1998 March;37(3):667-75.

148 Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T. Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. *Life Sci* 1998;62(26):2425-30.

149 Saitoh M, Osanai T, Kamada T et al. High plasma level of asymmetric dimethylarginine in patients with acutely exacerbated congestive heart failure: role in reduction of plasma nitric oxide level. *Heart Vessels* 2003 September;18(4):177-82.

150 Duckelmann C, Mittermayer F, Haider DG, Altenberger J, Wolzt M. Plasma asymmetric dimethylarginine and cardiovascular events in patients with acute decompensated heart failure. *Transl Res* 2008 July;152(1):24-30.

151 Nijveldt RJ, Teerlink T, van der HB et al. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. *Clin Nutr* 2003 February;22(1):23-30.

152 O'Dwyer MJ, Dempsey F, Crowley V, Kelleher DP, McManus R, Ryan T. Septic shock is correlated with asymmetrical dimethyl arginine levels, which may be influenced by a polymorphism in the dimethylarginine dimethylaminohydrolase II gene: a prospective observational study. *Crit Care* 2006;10(5):R139.

153 Richir MC, Ellger B, Teerlink T et al. The effect of rosiglitazone on asymmetric dimethylarginine (ADMA) in critically ill patients. *Pharmacol Res* 2009 December;60(6):519-24.

154 Nijveldt RJ, Teerlink T, van Leeuwen PA. The asymmetrical dimethylarginine (ADMA)-multiple organ failure hypothesis. *Clin Nutr* 2003 February;22(1):99-104.

155 Leiper J, Nandi M, Torondel B et al. Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat Med* 2007 February;13(2):198-203.

156 Asahara T, Murohara T, Sullivan A et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997 March 14;275(5302):964-7.

157 Hill JM, Zalos G, Halcox JP et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003 February 13;348(7):593-600.

158 Vasa M, Fichtlscherer S, Aicher A et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001 August 6;89(1):E1-E7.

159 Eizawa T, Ikeda U, Murakami Y et al. Decrease in circulating endothelial progenitor cells in patients with stable coronary artery disease. *Heart* 2004 July;90(6):685-6.

160 Tepper OM, Galiano RD, Capla JM et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002 December 26;106(22):2781-6.

161 Fadini GP, Miorin M, Facco M et al. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. *J Am Coll Cardiol* 2005 May 3;45(9):1449-57.

162 Loomans CJ, de Koning EJ, Staal FJ et al. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 2004 January;53(1):195-9.

163 Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH, Zhu JH. Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. *Clin Sci (Lond)* 2004 September;107(3):273-80.

164 Kondo T, Hayashi M, Takeshita K et al. Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arterioscler Thromb Vasc Biol* 2004 August;24(8):1442-7.

165 Choi JH, Kim KL, Huh W et al. Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 2004 August;24(7):1246-52.

166 Werner N, Kosiol S, Schiegl T et al. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005 September 8;353(10):999-1007.

167 Schmidt-Lucke C, Rossig L, Fichtlscherer S et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. *Circulation* 2005 June 7;111(22):2981-7.

168 Kalka C, Masuda H, Takahashi T et al. Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. *Proc Natl Acad Sci U S A* 2000 April 28;97(7):3422-7.

169 Shi Q, Rafii S, Wu MH et al. Evidence for circulating bone marrow-derived endothelial cells. *Blood* 1998 July 15;92(2):362-7.

170 Orlic D. Stem cell repair in ischemic heart disease: an experimental model. *Int J Hematol* 2002 August;76 Suppl 1:144-5.

171 Orlic D, Kajstura J, Chimenti S et al. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci U S A* 2001 August 28;98(18):10344-9.

172 Kawamoto A, Gwon HC, Iwaguro H et al. Therapeutic potential of ex vivo expanded endothelial progenitor cells for myocardial ischemia. *Circulation* 2001 February 6;103(5):634-7.

173 Kawamoto A, Tkebuchava T, Yamaguchi J et al. Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation* 2003 January 28;107(3):461-8.

174 Kocher AA, Schuster MD, Szabolcs MJ et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001 April;7(4):430-6.

175 Kaushal S, Amiel GE, Guleserian KJ et al. Functional small-diameter neovessels created using endothelial progenitor cells expanded ex vivo. *Nat Med* 2001 September;7(9):1035-40.

176 Takahashi M, Nakamura T, Toba T, Kajiwara N, Kato H, Shimizu Y. Transplantation of endothelial progenitor cells into the lung to alleviate pulmonary hypertension in dogs. *Tissue Eng* 2004 May;10(5-6):771-9.

177 Hirschi KK, Ingram DA, Yoder MC. Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2008 September;28(9):1584-95.

178 Hur J, Yoon CH, Kim HS et al. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. *Arterioscler Thromb Vasc Biol* 2004 March;24(2):288-93.

179 Ingram DA, Mead LE, Tanaka H et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 2004 November 1;104(9):2752-60.

180 Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest* 2000 February;105(1):71-7.

181 Gulati R, Jevremovic D, Peterson TE et al. Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. *Circ Res* 2003 November 28;93(11):1023-5.

182 Yoder MC, Mead LE, Prater D et al. Re-defining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* 2006 October 19.

183 Rehman J, Li J, Orschell CM, March KL. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003 April 4;107(8):1164-9.

184 Grunewald M, Avraham I, Dor Y et al. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006 January 13;124(1):175-89.

185 Pula G, Mayr U, Evans C et al. Proteomics identifies thymidine phosphorylase as a key regulator of the angiogenic potential of colony-forming units and endothelial progenitor cell cultures. *Circ Res* 2009 January 2;104(1):32-40.

186 Urbich C, Aicher A, Heeschen C et al. Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J Mol Cell Cardiol* 2005 November;39(5):733-42.

187 Sieveking DP, Buckle A, Celermajer DS, Ng MK. Strikingly different angiogenic properties of endothelial progenitor cell subpopulations: insights from a novel human angiogenesis assay. *J Am Coll Cardiol* 2008 February 12;51(6):660-8.

188 Au P, Daheron LM, Duda DG et al. Differential in vivo potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. *Blood* 2008 February 1;111(3):1302-5.

189 Melero-Martin JM, Khan ZA, Picard A, Wu X, Paruchuri S, Bischoff J. In vivo vasculogenic potential of human blood-derived endothelial progenitor cells. *Blood* 2007 June 1;109(11):4761-8.

190 Reinisch A, Hofmann NA, Obenauf AC et al. Humanized large-scale expanded endothelial colony-forming cells function in vitro and in vivo. *Blood* 2009 June 25;113(26):6716-25.

191 Orlic D, Kajstura J, Chimenti S et al. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001 April 5;410(6829):701-5.

192 Lipinski MJ, Biondi-Zoccai GG, Abbate A et al. Impact of intracoronary cell therapy on left ventricular function in the setting of acute myocardial infarction: a collaborative systematic review and meta-analysis of controlled clinical trials. *J Am Coll Cardiol* 2007 October 30;50(18):1761-7.

193 Abdel-Latif A, Bolli R, Tleyjeh IM et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch Intern Med* 2007 May 28;167(10):989-97.

194 Reffelmann T, Konemann S, Kloner RA. Promise of blood- and bone marrow-derived stem cell transplantation for functional cardiac repair: putting it in perspective with existing therapy. *J Am Coll Cardiol* 2009 January 27;53(4):305-8.

195 Heeschen C, Lehmann R, Honold J et al. Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 2004 April 6;109(13):1615-22.

196 Sasaki K, Heeschen C, Aicher A et al. Ex vivo pretreatment of bone marrow mononuclear cells with endothelial NO synthase enhancer AVE9488 enhances their functional activity for cell therapy. *Proc Natl Acad Sci U S A* 2006 September 26;103(39):14537-41.

197 Zhang Y, Herbert BS, Rajashekhar G et al. Premature senescence of highly proliferative endothelial progenitor cells is induced by tumor necrosis factor-alpha via the p38 mitogen-activated protein kinase pathway. *FASEB J* 2009 May;23(5):1358-65.

198 Yamaguchi J, Kusano KF, Masuo O et al. Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. *Circulation* 2003 March 11;107(9):1322-8.

199 Young PP, Hofling AA, Sands MS. VEGF increases engraftment of bone marrow-derived endothelial progenitor cells (EPCs) into vasculature of newborn murine recipients. *Proc Natl Acad Sci U S A* 2002 September 3;99(18):11951-6.

200 Zemani F, Silvestre JS, Fauvel-Lafeve F et al. Ex vivo priming of endothelial progenitor cells with SDF-1 before transplantation could increase their proangiogenic potential. *Arterioscler Thromb Vasc Biol* 2008 April;28(4):644-50.

201 Ceradini DJ, Kulkarni AR, Callaghan MJ et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004 September;10(8):858-64.

202 Kato J, Tsuruda T, Kita T, Kitamura K, Eto T. Adrenomedullin: a protective factor for blood vessels. *Arterioscler Thromb Vasc Biol* 2005 December;25(12):2480-7.

203 Kato H, Shichiri M, Marumo F, Hirata Y. Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cells. *Endocrinology* 1997 June;138(6):2615-20.

204 Kim W, Moon SO, Sung MJ et al. Angiogenic role of adrenomedullin through activation of Akt, mitogen-activated protein kinase, and focal adhesion kinase in endothelial cells. *FASEB J* 2003 October;17(13):1937-9.

205 Miyashita K, Itoh H, Sawada N et al. Adrenomedullin promotes proliferation and migration of cultured endothelial cells. *Hypertens Res* 2003 February;26 Suppl:S93-S98.

206 Iwase T, Nagaya N, Fujii T et al. Adrenomedullin enhances angiogenic potency of bone marrow transplantation in a rat model of hindlimb ischemia. *Circulation* 2005 January 25;111(3):356-62.

207 Kong XQ, Wang LX, Yang CS, Chen SF, Xue YZ, Liu YH. Effects of adrenomedullin on the cell numbers and apoptosis of endothelial progenitor cells. *Clin Invest Med* 2008;31(3):E117-E122.

208 Abe M, Sata M, Nishimatsu H et al. Adrenomedullin augments collateral development in response to acute ischemia. *Biochem Biophys Res Commun* 2003 June 20;306(1):10-5.

209 Fujii T, Nagaya N, Iwase T et al. Adrenomedullin enhances therapeutic potency of bone marrow transplantation for myocardial infarction in rats. *Am J Physiol Heart Circ Physiol* 2005 March;288(3):H1444-H1450.

210 Iimuro S, Shindo T, Moriyama N et al. Angiogenic effects of adrenomedullin in ischemia and tumor growth. *Circ Res* 2004 August 20;95(4):415-23.

211 Oehler MK, Hague S, Rees MC, Bicknell R. Adrenomedullin promotes formation of xenografted endometrial tumors by stimulation of autocrine growth and angiogenesis. *Oncogene* 2002 April 25;21(18):2815-21.

212 Hasdai D, Lerman A. The assessment of endothelial function in the cardiac catheterization laboratory in patients with risk factors for atherosclerotic coronary artery disease. *Herz* 1999 November;24(7):544-7.

213 Corretti MC, Anderson TJ, Benjamin EJ et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002 January 16;39(2):257-65.

214 Kuvin JT, Patel AR, Sliney KA et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2003 July;146(1):168-74.

215 Hamburg NM, Palmisano J, Larson MG et al. Relation of brachial and digital measures of vascular function in the community: the Framingham heart study. *Hypertension* 2011 March;57(3):390-6.

216 Dhindsa M, Sommerlad SM, DeVan AE et al. Interrelationships among noninvasive measures of postischemic macro- and microvascular reactivity. *J Appl Physiol* 2008 August;105(2):427-32.

217 Nohria A, Gerhard-Herman M, Creager MA, Hurley S, Mitra D, Ganz P. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol* 2006 August;101(2):545-8.

218 Joannides R, Haefeli WE, Linder L et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation* 1995 March 1;91(5):1314-9.

219 Loscalzo J, Vita JA. Ischemia, hyperemia, exercise, and nitric oxide. Complex physiology and complex molecular adaptations. *Circulation* 1994 November;90(5):2556-9.

220 Engelke KA, Halliwill JR, Proctor DN, Dietz NM, Joyner MJ. Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm. *J Appl Physiol* 1996 October;81(4):1807-14.

221 Tschakovsky ME, Pyke KE. Counterpoint: Flow-mediated dilation does not reflect nitric oxide-mediated endothelial function. *J Appl Physiol* 2005 September;99(3):1235-7.

222 Green D. Point: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. *J Appl Physiol* 2005 September;99(3):1233-4.

223 Pyke KE, Tschakovsky ME. The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function. *J Physiol* 2005 October 15;568(Pt 2):357-69.

224 Bishop ET, Bell GT, Bloor S, Broom IJ, Hendry NF, Wheatley DN. An in vitro model of angiogenesis: basic features. *Angiogenesis* 1999;3(4):335-44.

225 Hochman JS, Sleeper LA, White HD et al. One-year survival following early revascularization for cardiogenic shock. *JAMA* 2001 January 10;285(2):190-2.

226 Kashani A, Giugliano RP, Antman EM et al. Severity of heart failure, treatments, and outcomes after fibrinolysis in patients with ST-elevation myocardial infarction. *Eur Heart J* 2004 October;25(19):1702-10.

227 Hochman JS, Sleeper LA, Webb JG et al. Early revascularization and long-term survival in cardiogenic shock complicating acute myocardial infarction. *JAMA* 2006 June 7;295(21):2511-5.

228 Berger PB, Tuttle RH, Holmes DR, Jr. et al. One-year survival among patients with acute myocardial infarction complicated by cardiogenic shock, and its relation to early revascularization: results from the GUSTO-I trial. *Circulation* 1999 February 23;99(7):873-8.

229 Vincent JL, Moreno R, Takala J et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996 July;22(7):707-10.

230 Chello M, Goffredo C, Patti G et al. Effects of atorvastatin on arterial endothelial function in coronary bypass surgery. *Eur J Cardiothorac Surg* 2005 December;28(6):805-10.

231 Morelos M, Amyot R, Picano E et al. Effect of coronary bypass and cardiac valve surgery on systemic endothelial function. *Am J Cardiol* 2001 February 1;87(3):364-6, A10.

232 Thijssen DH, de Groot PC, Kooijman M, Smits P, Hopman MT. The sympathetic nervous system contributes to the age-related impairment of flow-mediated dilation of the superficial femoral artery. *Am J Physiol Heart Circ Physiol* 2006 July 14.

233 Quayle JM, Nelson MT, Standen NB. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev* 1997 October;77(4):1165-232.

234 Berger R, Stanek B, Hulsmann M et al. Effects of endothelin a receptor blockade on endothelial function in patients with chronic heart failure. *Circulation* 2001 February 20;103(7):981-6.

235 Astiz ME, DeGent GE, Lin RY, Rackow EC. Microvascular function and rheologic changes in hyperdynamic sepsis. *Crit Care Med* 1995 February;23(2):265-71.

236 Giannattasio C, Piperno A, Failla M, Vergani A, Mancia G. Effects of hematocrit changes on flow-mediated and metabolic vasodilation in humans. *Hypertension* 2002 July;40(1):74-7.

237 Matsumoto M, Tsujino T, Lee-Kawabata M et al. Serum interleukin-6 and C-reactive protein are markedly elevated in acute decompensated heart failure patients with left ventricular systolic dysfunction. *Cytokine* 2010 March;49(3):264-8.

238 de W, I, Jaccard C, Corradin SB et al. Cytokines, nitrite/nitrate, soluble tumor necrosis factor receptors, and procalcitonin concentrations: comparisons in patients with septic shock, cardiogenic shock, and bacterial pneumonia. *Crit Care Med* 1997 April;25(4):607-13.

239 Verrier ED, Boyle EM, Jr. Endothelial cell injury in cardiovascular surgery. *Ann Thorac Surg* 1996 September;62(3):915-22.

240 Paparella D, Yau TM, Young E. Cardiopulmonary bypass induced inflammation: pathophysiology and treatment. An update. *Eur J Cardiothorac Surg* 2002 February;21(2):232-44.

241 Loukanov T, Arnold R, Gross J et al. Endothelin-1 and asymmetric dimethylarginine in children with left-to-right shunt after intracardiac repair. *Clin Res Cardiol* 2008 February 22.

242 Karu I, Zilmer K, Starkopf J, Zilmer M. Changes of plasma asymmetric dimethylarginine levels after coronary artery bypass grafting. *Scand Cardiovasc J* 2006 December;40(6):363-7.

243 Tschaikowsky K, Neddermeyer U, Pscheidl E, von der EJ. Changes in circulating blood volume after cardiac surgery measured by a novel method using hydroxyethyl starch. *Crit Care Med* 2000 February;28(2):336-41.

244 Hoeft A, Schorn B, Weyland A et al. Bedside assessment of intravascular volume status in patients undergoing coronary bypass surgery. *Anesthesiology* 1994 July;81(1):76-86.

245 Fernandez-Sauze S, Delfino C, Mabrouk K et al. Effects of adrenomedullin on endothelial cells in the multistep process of angiogenesis: involvement of CRLR/RAMP2 and CRLR/RAMP3 receptors. *Int J Cancer* 2004 March 1;108(6):797-804.

246 Guidolin D, Albertin G, Spinazzi R et al. Adrenomedullin stimulates angiogenic response in cultured human vascular endothelial cells: Involvement of the vascular endothelial growth factor receptor 2. *Peptides* 2008 November;29(11):2013-23.

247 Schwarz N, Renshaw D, Kapas S, Hinson JP. Adrenomedullin increases the expression of calcitonin-like receptor and receptor activity modifying protein 2 mRNA in human microvascular endothelial cells. *J Endocrinol* 2006 August;190(2):505-14.

248 He T, Peterson TE, Katusic ZS. Paracrine mitogenic effect of human endothelial progenitor cells: role of interleukin-8. *Am J Physiol Heart Circ Physiol* 2005 August;289(2):H968-H972.

249 Zhang Y, Ingram DA, Murphy MP et al. Release of proinflammatory mediators and expression of proinflammatory adhesion molecules by endothelial progenitor cells. *Am J Physiol Heart Circ Physiol* 2009 May;296(5):H1675-H1682.

250 Sironi M, Breviario F, Proserpio P et al. IL-1 stimulates IL-6 production in endothelial cells. *J Immunol* 1989 January 15;142(2):549-53.

251 Fee D, Grzybicki D, Dobbs M et al. Interleukin 6 promotes vasculogenesis of murine brain microvessel endothelial cells. *Cytokine* 2000 June;12(6):655-65.

252 Koch AE, Polverini PJ, Kunkel SL et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* 1992 December 11;258(5089):1798-801.

253 Li A, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J Immunol* 2003 March 15;170(6):3369-76.

254 Yao JS, Zhai W, Young WL, Yang GY. Interleukin-6 triggers human cerebral endothelial cells proliferation and migration: the role for KDR and MMP-9. *Biochem Biophys Res Commun* 2006 April 21;342(4):1396-404.

255 Fan Y, Ye J, Shen F et al. Interleukin-6 stimulates circulating blood-derived endothelial progenitor cell angiogenesis in vitro. *J Cereb Blood Flow Metab* 2008 January;28(1):90-8.

11. PAPERS

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APPENDIX

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