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Neuroendocrine Regulation of Stress Response in Clinical Models

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<http://dx.doi.org/10.5772/48533>

1. Introduction

A stress response is an evolutionary heritage of ability to anticipate, identify and effectively respond to danger. After millions of years of evolution, perception of variety of stressors mobilizes neurologic, neuroendocrine, endocrine, immunologic and metabolic systems to maintain an ability to survive and propagate genes (natural selection). Additionally, in humans these mechanisms involve complex and interrelated mental, emotional, behavioral and social processes. Behavioral adaptation is aimed on modulation of neural pathways that help to cope with stressful situations. These e.g. include changes of sensory thresholds, increased alertness, memory enhancement, suppression of hunger, and stress-induced analgesia.

A stressor can be defined as a certain stimulus of the external or internal receptor. The stressors are usually divided into macroscopic threats (e.g. fight with enemy, fear, pain) and microscopic threats (targeting at epithelial or endothelial barriers e.g. infection or tissue damage). These neuroendocrine – immunologic interrelations are also vital in the clinical situations. During an acute stress response, physiological processes are aimed on redistribution of energy utilization in specific organs, inhibiting or stimulating energy mobilization. Therefore certain tissues receive sufficient supply of energy while others reduce their consumption according to priority. This is achieved mainly by: the sympathetic nervous system (SNS), release of catecholamines which inhibit insulin release and action, stimulates glucagon and ACTH production; hypothalamic – pituitary – adrenocortical (HPA) axis that in general increases gluconeogenesis and glycogenolysis, inhibits glucose uptake, and enhances proteolysis and lipolysis; hypothalamic - posterior pituitary (ADH) – kidney axis with water retention; brain – juxta-glomerular apparatus activity - (renin/angiotensin/aldosterone - RAAS) with many effects on blood pressure, electrolytes and water balance; hypothalamic-pituitary-thyroid axis (response to cold and heat), natriuretic

peptides, the parasympathetic nervous system (acetylcholine release), changes in immune system (cytokines and other pro-inflammatory substances), mediators of endothelial function and mobilization of stem cells.

In the clinical settings, variety of interesting models and complex relations can be investigated. In particular, pathophysiology and treatment of congenital heart defects create unique models of stress response. Hypoxia, circulatory insufficiency, volume or pressure overload, hypo- or hyperthermia, pain, changes in organ perfusion, disturbances of the osmolarity, inflammatory- or immune- response create exceptional milieu and environment for the research.

In this chapter we reviewed main concepts of stress response in such environment additionally presenting some results of own research. It focuses on patients who had strong stressors working in acute or chronic manner (desaturation, increased afterload, volume overload, circulatory insufficiency) with all related elements affecting the model in clinical environment.

2. The arrangement of the stress response

The stress response is the complex process that can be initiated by immune or central nervous system. The central nervous system reacts against macroscopic threats and controls whole body response. Thus, in face of lacking of the system integrity central nervous system switches all functions over to subordinate constitutive activities to defense against the threat. The hypothalamus – pituitary – adrenal axis is activated and vasopressin, prolactin and growth hormone are released. In clinical settings corticotropin realizing hormone and vasopressin (both stimulated by adreno-cortical signals e.g. pain, fear, hypovolemia or immunologic stimuli e.g. interleukins, TNF, cytokines) (1) synergistically increase adrenocorticotropin (ACTH) secretion. ACTH induces conversion of cholesterol to cortisol which cooperates with sympathetic nervous system to prepare a body for response by mobilization of energetic substrates, increase of intravascular volume and blood pressure enhancement (Tab.1.).

The immune system reacts against microscopic threats infringing endothelial or epithelial barriers. The initial signal is amplified by cascade of lymphokines and activated cells and stimulates central stress response which eventually terminates system overstimulation. Immune response and tissue damage contribute to systemic inflammatory response syndrome (SIRS) development. These inflammatory signals are transferred to the central nervous system by vagus nerve and activate HPA axis (2).

Adaptation to chronic stress in humans is not well understood and unnatural situation. It is mostly created in the clinical settings when treatment of critical disease is implemented and it reaches chronic phase. After acute stress response when ACTH, prolactin, growth hormone, and thyroid hormone are elevated, the pulsatile, more physiologic pattern of neurohormones concentration appears. Although normal limits of plasma neurohormons levels in stress response are not known, inadequate concentrations can lead to acute failure and shock.

Action	Mechanism
Growth inhibition	Decrease of DNA and RNA synthesis Increase of protein catabolism in all tissues Enhancement of protein synthesis in the liver
Substrates availability increase	Glycolysis, lipolysis, protein hydrolysis
Blood pressure increase	Vascular tone increase, Expression of adrenergic receptors Activation of renin – angiotensin – aldosterone system
Inhibition of constitutive functions	Suppression of immune response Decrease of circulating lymphocytes, monocytes and eosinophils Apoptosis induction
Anti-inflammatory	Decrease of capillary permeability, phagocytosis, leucocyte demargination, interleukine synthesis
Water balance	Sodium and water reabsorbtion

Table 1. Role of the cortisol in stress response initiation.

3. Sympathetic nervous system

Sympathetic nervous system stimulation is a part of central regulatory mechanism. It exerts many effects on the cardiovascular system by norepinephrine and epinephrine. Afferent baroreceptor signaling to the brain signals low cardiac output and efferent sympathetic pathways are activated. The main results of it are vasoconstriction (increased afterload, decreased renal perfusion), increased heart rate and contractility (increased cardiac output and wall stress), activation of RAAS. These effects are aimed on restoration of cardiac output, however, at the expense of increased myocardial oxygen demand, increased intracellular calcium toxicity, and myocardial hypertrophy. Sympathetic overstimulation can cause many undesirable effects like: expression of fetal gens, apoptosis, necrosis and remodeling and high levels of plasma norepinephrine are an independent predictor of mortality.

In clinical model of univentricular circulation characterized by increased afterload and normal saturation interesting behavioral adaptation was observed. During the exercise the heart rate at anaerobic threshold was significantly slower and patients' lung tidal volume lower compared to healthy age matched volunteers. These differences disappeared at peak effort. The effect was associated with a delayed chronotropic response of the heart and a reaction which provides a longer filling time and larger preload to the single ventricle. Delayed chronotropic response, earlier achievement of anaerobic threshold and higher value of ventilator equivalent of carbon dioxide at peak exercise obviously reflect greater impairment of cardiac output in single ventricle patients compared to healthy volunteers. The limitation of the exercise capacity is

caused mainly by abnormal autonomic nervous system activity, lower non-pulsatile pulmonary flow, neurohormonal disturbances and dysfunction of the endothelium. The primary mechanism restricting exercise capacity is the lack of ability to increase and maintain the cardiac output and pulmonary flow in response to exercise. This is complementary with delayed chronotropic reaction, decreased heart rate acceleration and abnormal reflex from ergoreceptors. Exercise studies with external pacemaker heart stimulation to increase heart rate despite of slowing it reflex did not cause increase of exercise tolerance (3). In our model heart rate was significantly lower at anaerobic threshold indicating delayed chronotropic response or adaptation to the demand of increased output generation (the slower the heart rate, the better preload). This was accompanied by significant respiratory tidal volume lowering, diminished carbon dioxide production, and respiratory equivalent of carbon dioxide compared to control group. These differences disappeared at peak exercise suggesting maintenance of optimal hemodynamic and respiratory parameters for maximal physiological effect.

Effects of sympathetic stimulation	Cellular effects
Heart	Increased cardiomyocyte calcium entry
Increased contractility (inotropy)	Myocardial hypertrophy
Increased heart rate	Gene expression:
Increased wall stress	Increased expression of fetal genes
Decreased myocardial relaxation (lusitropy)	Decreased expression of calcium metabolism genes
Increased oxygen demand	Apoptosis
Peripheral vessels	Necrosis
Constriction	Fibrosis
Increased afterload	Myocardial hypertrophy / remodeling
Kidney	β 1 - receptors down-regulation
Vasoconstriction	
Sodium retention	
Water retention	
RAAS activation	
Sodium retention	
Water retention	

Table 2. Sympathetic nervous system activation

Significant positive correlation of VE/VCO₂ (respiratory equivalent of carbon dioxide) at peak exercise with proBNP and endothelin-1 were found. The parameter VE/VCO₂ reflects relationship between minute ventilation and carbon dioxide clearance and is considered as a more sensitive prognostic factor than oxygen consumption in diagnosis of circulatory insufficiency. In patients with chronic heart failure VE/VCO₂ is increased and negatively correlated with cardiac output at peak exercise and is independent of subject effort and peripheral function (3, 4). In our study VE/VCO₂ peak is significantly higher in investigated group, compared to age matched controls. The correlation with endothelin-1 and proBNP indicates the possibility of identification of such patients by neurohormonal screening tests

and suggests etiology of such condition. Higher concentration of endothelin-1 reveals endothelial dysfunction and can contribute to higher resistance of pulmonary vascular bed and lower pulmonary blood flow at peak exercise. Higher BNP concentrations can indicate more pronounced ventricular dysfunction (5).

4. Hypothalamic-pituitary-adrenal axis

Hypothalamic – pituitary – adrenal system is the central stress response system linking neural regulation to neurohormonal and humoral control. In response to cortical signals e.g. fear, pain, deep emotions or immune derived factors like TNF α , IL-6 corticotropin releasing hormone, vasopressin, prolactin and growth hormone are released. Corticotropin releasing hormone stimulates sympathetic system and ACTH secretion. It reaches the adrenal cortex and stimulates cortisol production from cholesterol. Cortisol cooperates with sympathetic activation to prepare metabolism for stress response. These mechanism inhibit all growth and developmental functions, prepare metabolic substrates (glucose, fatty acids, amino acids), increase blood pressure and intravascular volume.

There is insufficiency of hypothalamic-pituitary-adrenal axis after pediatric cardiac surgery observed, best described as a critical illness–related corticosteroid insufficiency (CIRCI). Together with other axes derangement it is considered as one of the causes of low cardiac output syndrome in postoperative period. Many causes of this phenomenon were proposed: brain hypoperfusion, central hypothalamus and pituitary gland insufficiency, tissue resistance to adrenocorticotrophic hormone (ACTH), adrenal dysfunction, cyanosis and tissues immaturities.

5. Endotheline

Endothelins (ET -1,-2,-3) are a molecules produced by endothelium acting as a vasoconstrictors and mitogenic factors. In patients with heart failure their plasma concentrations are increased their concentration is proportional to the severity of the disease. Endothelins promote vasoconstriction, inflammation, fibrosis, and hypertrophy in the pulmonary and systemic vasculature.

Plasma ET-1 levels are elevated in patients who have cardiomyopathy or chronic heart failure, and correlate with severity and prognosis. In particular, the degree of plasma elevation of endothelin correlates with the magnitude of alterations in cardiac hemodynamics and functional class.

In our material, higher pulmonary artery resistance was related to higher endothelin concentration in patients with single ventricle, therefore endothelin receptor antagonist could result in reduction of pulmonary resistance.

6. Renin angiotensin aldosterone axis

Renin angiotensin aldosterone axis exerts many effects in cardiovascular system. Neural connexion of the brain and kidneys is stimulated by low sodium, decreased perfusion,

increased alpha – adrenergic activity. It can effect juxtaglomerular apparatus increasing renin - protease transforming angiotensinogen to angiotensin I which is converted within the endothelial cells (particularly concentrated in the lungs) to angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II is the most potent vasoconstrictor increasing vascular resistance in stress situations (especially in hypovolemia) and effecting adrenal cortex increasing aldosterone production which increases reclaiming of sodium and water. And its major role is to maintain the circulating volume status.

7. Vasopressin system

Vasopressin (ADH) is released by the hypothalamus as a result of baroreceptor, osmotic, and neurohormonal stimuli. It normally maintains body fluid balance, vascular tone, and regulates contractility. Heart failure causes a paradoxical increase in AVP. The increased blood volume and atrial pressure in heart failure suggest inhibition of vasopressin secretion, but it does not occur. This phenomenon is related to SNS and RAAS activation overriding the volume and low-pressure cardiovascular receptors and osmotic vasopressin regulation causing increase in AVP secretion. It contributes to the increased systemic vascular resistance (V1 receptors) and to renal retention of fluid (V2 receptors). Stimulation of V1 receptors can also cause vasoconstriction of the peripheral vessels, platelet aggregation, and adrenocorticotrophic hormone stimulation. Low-dose arginine infusion initiated in the operating room after complex neonatal cardiac surgery was associated with decreased fluid resuscitation and catecholamine. The vasopressin levels are usually high in the early phase of septic shock, but it's deficiency was noted in vasodilatory shock.

The important mechanism of vasopressin action in stress states is its potentiating effect on ACTH secretion leading to cortisol release. Although vasopressin is a powerful vasoconstrictor it dilates the pulmonary, cerebral, and myocardial circulations helping to preserve vital organ blood flow.

In our group of patients with single ventricle, there was a significant correlation between vasopressin concentration and disturbances of water – electrolyte balance in single ventricle patients. Higher vasopressin plasma levels were connected with greater propensity for fluid retention and prolonged pleural effusions (6).

8. Thyroid hormones

Thyroid hormones are stimulated by TSH anterior pituitary secretion. There is many actions of thyroid hormones on cardiovascular system exerted mainly by triiodothyronine (T3). These effects can be divided into genomic and extragenomic actions. T3 bounds to the nuclear receptors and activates many genes corresponding to key myocardial functions: myosin heavy chain (MHC), sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA2) and its inhibitor phospholamban (affecting cardiac contractile function and diastolic relaxation), voltage-gated K^{+} channels, β_1 -adrenergic receptor, guanine nucleotide regulatory proteins, adenylate cyclase, $\text{Na}^{+}/\text{K}^{+}$ -ATPase, and Na/Ca exchanger. The main cardiovascular effects

of T3 are: increased cardiac contractility, reduction of afterload, reduction of vascular resistance, chronotropic effect (increased heart rate), increases sodium reabsorption and water improves atrial filling pressure. All of this increase cardiac output.

T3 has genomic effects that maintain endothelial integrity, such as angiotensin receptors in vascular smooth muscle cells (VSMC). This supports the hypothesis that the vasculature is a principal target for T3 action. T3 decreases resistance in peripheral arterioles. Extragenomic actions include: modulation of cellular metabolic activities, such as glucose and amino acid transport, ion fluxes at the level of the plasma membrane, and mitochondrial gene expression and function.

9. Cholinergic pathway

Together with the stimulation of the adrenergic system the feedback is also started as anti-inflammatory cholinergic pathway. It is comprised of vagus nerve signals leading to acetylcholine interaction with receptors on monocytes and macrophages, resulting in reduced cytokine production. It can prevent tissue injury and improve survival by external stimulation. The cholinergic anti-inflammatory pathway exerts a tonic, inhibitory influence on immune responses to infection and tissue injury. Interrupting this pathway, produces exaggerated responses to bacterial products and injury.

10. Natriuretic peptides

Natriuretic peptide system counteracts of some of the effects of neurohormonal activation causing vasodilatation, reduction of aldosterone production (by direct influence on the adrenal gland), increased diuresis and natriuresis, reduction of renin production, decreased vasopressin release, decreased activation of the sympathetic nervous system. Direct influence of the natriuretic peptides on the myocardium includes prevention of hypertrophy and reduction of fibroblast proliferation. BNP is a natriuretic peptide released in response to ventricular volume expansion and pressure overload.

Cardiopulmonary by-pass in children induces renal and neurohormonal changes similar to those observed in congestive heart failure: upregulation of the RAA axis, increase of renin concentration, release of vasopressin. The endogenous biological activity of natriuretic hormone system is decreased after the bypass. This is caused by deficiency of biologically active neurohormones, presence of inactive neurohormones, resistance to natriuretic hormone activity, receptor down-regulation, abnormal signal transduction, increased phosphodiesterase activity.

It has been also shown that neurohormones can decrease ischemia-reperfusion injury in multiple tissue including heart by inhibition of angiotensin II and aldosterone, limitation of intracellular Ca^{++} overload, maintenance of ATP stores, preservation of myofibril, mitochondrial and nuclear structure of cardiomyocytes.

In the natural history of diseased cardiovascular system complex interactions between local, humoral, and neural factors lead to abnormalities in the circulatory control. These adaptive

responses are aimed at maintaining adequate vital organ perfusion but can lead to unfavorable and undesirable changes both in the heart and the vascular system. An impaired regulation of cardiac autonomic system and activation of many neurohormonal factors as well as the rennin-angiotensin-aldosterone system (RAAS). These changes may contribute to numerous early and late complications e.g. dysregulation of fluid homeostasis, effusions, detrimental remodeling, protein-losing enteropathy and limited exercise capacity. They can also serve as important indices for risk stratification, prediction of unfavorable events and adjustment of treatment (3).

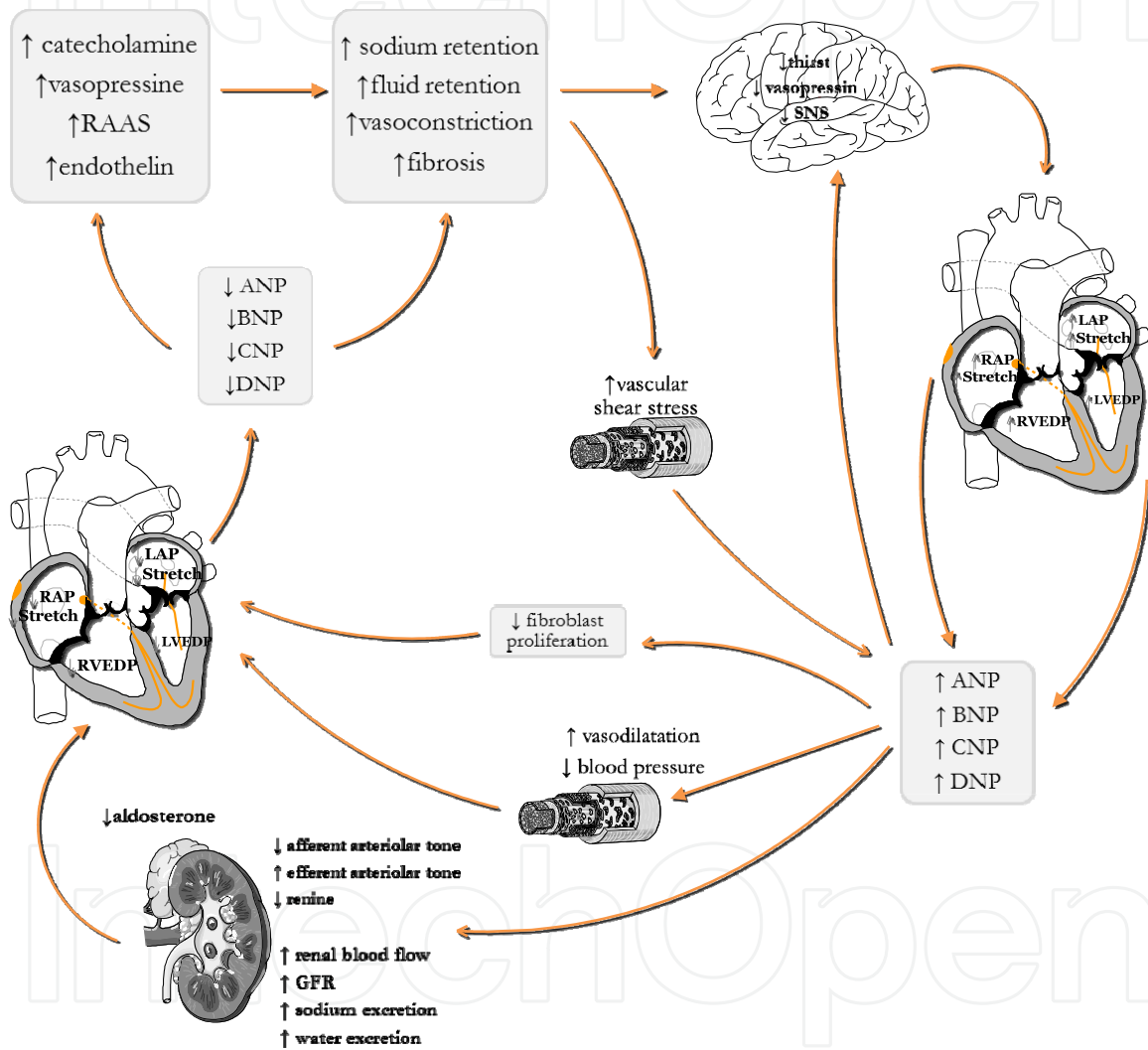


Figure 1. Natruretic factors interactions.

11. Stem cells

Stem cells are specific cells with ability to unlimited divisions and differentiation. There are many types of stem cells depending on the differentiation degree. Residual small cells with embryonic stem cells phenotype (*VSEs*, *Very Small Embryonic-like Cells*) are a population of pluripotent cells deposited in developing organs during embryogenesis. In the bone marrow

VSELs find beneficial conditions to growth and become reserve cell line participating in tissue and organ regeneration. In the postnatal life they are inactive and flow in blood stream in small amount. Mobilization of VSEL's is considered as a part of stress response it can increase upon different impulses e.g. tissue damage, ischemia, hypoxia, myocardial infarction, open heart surgery, extracorporeal circulation. Cells mobilized from bone marrow penetrate to blood and are attracted to damaged tissues by chemotactic factors, e.g. SDF-1, HGF/SF, or VSEGF.

Researches who identified and described morphology of VSELs also showed the ability of those cells to proliferate and differentiate into all three primary germ layers in appropriate differentiating medium. It has been also proved that VSELs express many markers of primordial germ cells, e.g. fetal alkaline phosphatase, Oct-4, SSEA-1, CXCR4, Mvh, Stella, Fragilis, Nobox and Hdac6, indicating their similarity to germ cells through which genes are passed from generation to generation – the best reservoir of stem cells (7, 8). Most active translocation of stem cells takes place during early stage of human embryogenesis. In the beginning of gastrulation and organogenesis stem cells migrate to places of new tissues and organs formation. Subsequently, stem cells settle down in tissue specific spaces and constitute a cell line undergoing self-renewal process. These cells also replenish damaged or apoptotic cells during individual life. VSELs may accumulate in bone marrow under the influence of chemotactic factors (correlation between CXCR4 receptor and lymphokine SDF-1). After colonizing bone marrow VSELs find beneficial conditions to growth and become reserve cell line participating in tissue and organ regeneration. In normal conditions VSELs circulate in the peripheral blood in small number and can increase upon different stimuli e.g. tissue damage or severe stress (ischemia, hypoxia, myocardial infarction, open heart surgery, extracorporeal circulation) (9). Cells mobilized from bone marrow penetrate to blood and are attracted to damaged tissues by chemotactic factors, e.g. SDF-1, HGF/SF, or VSEGF. It has been proved that many clinical scenarios are associated with increase of stem cells in bloodstream. Increase of the number of bone marrow derived stem cells was observed in skeletal muscle injury, myocardial infarction, stroke, bones fractures, lesions of the liver and kidneys, ischaemia of the extremities and after lung or liver transplantation. These cells were described as endothelial progenitor cells (EPC), myocardial or muscle progenitor cells, neural progenitor cells, liver progenitor cells etc... These data indicate that during injury of the tissues and organs non-hematopoietic stem cells are mobilized from the marrow (10) and probably from other tissue niches to the blood where they circulate as a source of the stem cells supporting regeneration of the tissues (11, 12). This process is governed by injured tissue derived chemoattractants such as SDF-1, and other factors e.g.: VEGF, HGF/SF, UF and FGF-2. It is also known that transcriptional factor HIF-1 (hypoxia regulated/induced transcription factor) connected with the tissue ischemia takes important place in regulation of expression of these factors. The promotor for sdf-1, vegf and hgf/sf gens have bounding places for HIF-1. Therefore hypoxia / cyanosis can induce expression of factors responsible for stem cells releasing and their migration to the injured tissues and organs. VSELs which are present in the marrow are quiescent and they need unknown factors for activation and stimulation of their activity. These incentives and modulators are unknown.

Recent research indicates that in the mature hearts of the mammals there is a population of the cells capable of mitotic divisions named cardiac stem cells (CSC). They are pluripotential, clonogenic, and self-replicable. Their location in the heart seems to be related to the mechanical load of given segment of the heart muscle and is inversely proportional to hemodynamic load. The number of CSC depends on the methodology of counting and ranges from 1/8000 to 1/20 000 cardiomyocytes or 1/ 32 000 – 1/80 000 all cells of the heart.

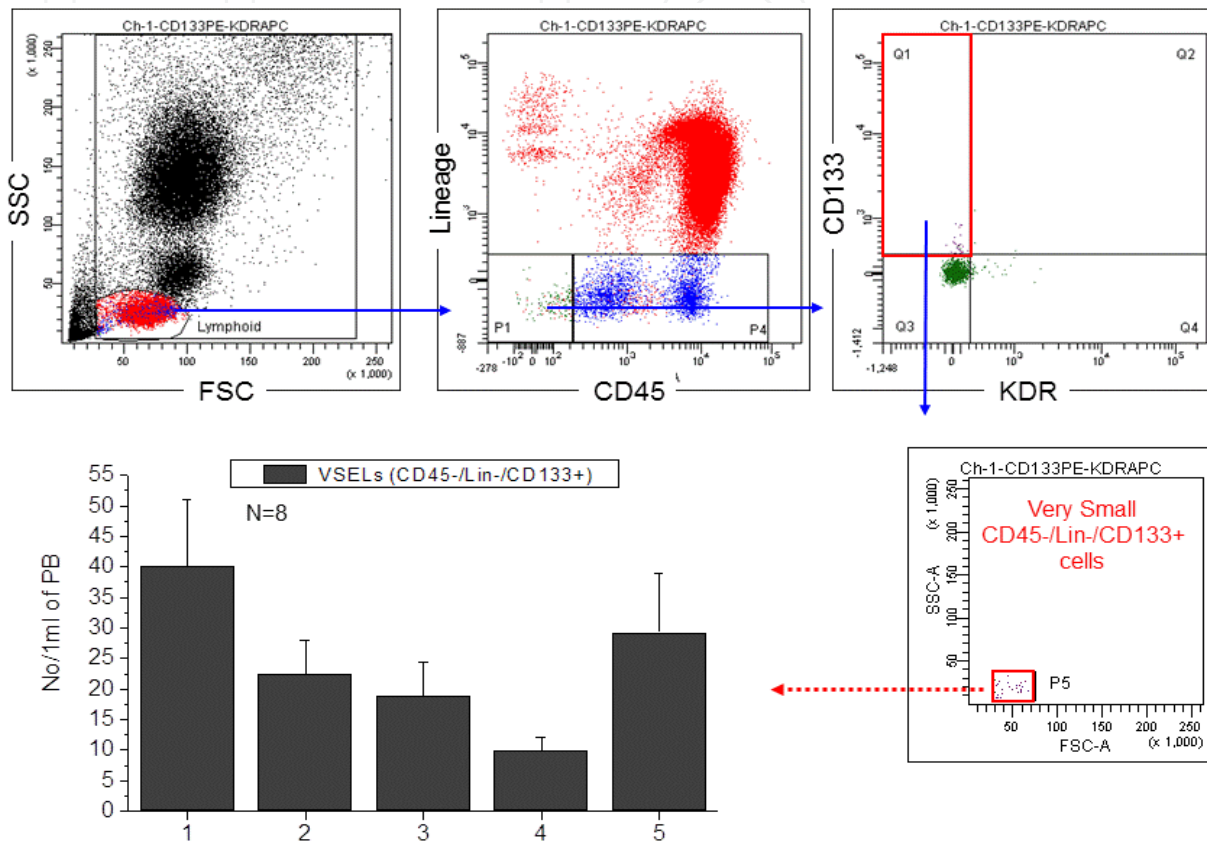


Figure 2. Flow cytometry – mobilization of VSELs in children undergoing heart surgery due to congenital heart diseases

In population of our patients we've obtained blood specimens before the operation and during the hospitalization to determine the level of VSELs mobilization. Using the flow cytometry it has been shown that VSELs appears in peripheral blood with a specified pattern of mobilization during surgery and directly after it (Fig.2.) and confirmed the presence of those cells within myocardium Fig.3.

The acute phase of stress response is characterized by increased release of neuroendocrine mediators from the hypothalamus and pituitary. This is aimed on the blood pressure maintenance and mobilization of fuel substrates at the expense of deregulation of homeostatic mechanisms, immunologic response, growth, development and regeneration. If stress response is insufficient to maintain tissue perfusion, shock appears.

During the prolonged phase of critical illness, the effects of the stress response mediators, may be harmful. Decreased levels of anterior pituitary hormones and loss of the normal

pattern of pulsatile release of these hormones characterize the prolonged phase of critical illness. Cortisol levels remain elevated in chronic critical illness despite a decrease in ACTH release. The metabolic result of this neuroendocrine array is worsen metabolism of fatty acids and a propensity for fat storing and protein wasting. The immune effects related to neuroendocrine disturbances are impaired lymphocyte and monocyte function and increased lymphocyte apoptosis. It leads to catabolic state and multiple organ dysfunction. Duration of immune suppression correlates strongly with the incidence of related infection. Tissue damage and strong stressors (such as cyanosis, circulatory insufficiency) stimulate regenerative and reparative processes involving stem cells.



Figure 3. Very small embryonic-like cells extracted from the heart

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Acknowledgement

This work is supported by government grant No 2011/01/B/NZ5/04246

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