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European Perspectives in Cardiology



Circulation: European Perspectives

Funding: The Slovenian Research Agency

“A Major Improvement in the Transparency of Funding Research Compared to the Way This Was Done in the Previous Totalitarian Regime”

Scientists who have received a grant from the Slovenian Research Agency to carry out cardiovascular research describe their work to Jennifer Taylor, BSc, MSc, MPhil.

The Slovenian Research Agency selects programmes and projects that will be funded by the government and other bodies. Based in the capital of Slovenia, Ljubljana, the Agency's highest decision-making body is the Scientific Council, which comprises 6 members representing different scientific disciplines. The president and members of the Scientific Council are nominated by the minister responsible for science. A number of projects and programmes are being funded in the area of cardiovascular science.

Investigating Microvesicles as a Mechanism Underlying Thromboembolic Disorders

Veronika Kralj-Iglič, PhD, professor of biophysics, Faculty of Health Sciences, University of Ljubljana, and Department of Orthopaedic Surgery, Ljubljana University Medical Centre, Ljubljana

Professor Kralj-Iglič received a 3-year grant of €150,000 starting in July 2011 to fund 1 staff member, chemicals,

publications, travel, and equipment. The title of her project is “Microvesicles as Risk Factors for Secondary Thromboembolic Events.” Professor Kralj-Iglič is the principal investigator and the Faculty of Health Sciences, University of Ljubljana, is the leading institution. Collaborators are based in the Faculty of Medicine, Biotechnical Faculty, and Faculty of Electrical Engineering at the University of Ljubljana; the National Institute of Chemistry; Ljubljana University Medical Centre; Abo Akademi University, Turku, Finland; Technical University, Prague, Czech Republic; and the Institute of Biophysics and Nanosystems Research, Austrian Academy of Science, Austria.

The focus of the project is microvesicles isolated from blood as diagnostic tools and basic mechanisms underlying thromboembolic disorders and cancer progression. Microvesiculation is a common process in all cells in which membrane-enclosed fragments from the cell interior are pinched off from the mother cell and become more or



Professor Kralj-Iglič and her research team. From left to right: R. Štukelj, PhD student; Professor D. Drobne, PhD; R. Janša, MD; Professor V. Kralj-Iglič, PhD, principal investigator; E. Ogorevc, PhD student; T. Slokar, PhD student; A. Bedina Zavec, PhD; V. Šuštar, PhD. Photograph courtesy of Marija Cerar.

less free to move in the surrounding solution. By interacting with distal cells, microvesicles constitute a cell-to-cell communication system and a catalytic surface for blood clot formation. Clinical studies have shown that the concentration of microvesicles isolated from blood is increased in patients with thromboembolism and different types of cancer.

Professor Kralj-Iglič says, “We hypothesise that increased microvesiculation could be connected to the increased risk for thromboembolism in cancer patients.”

Since the project started, Professor Kralj-Iglič and her colleagues have published an article revealing the identity and mechanisms of the formation of microvesicles isolated from blood,¹ as well as an article considering quantification of mediated interaction between membranes.²

“Within the grant, we hope to improve the understanding of the budding and vesiculation processes and their role in thromboembolism secondary to cancer,” says Professor Kralj-Iglič. “Also, we would like to improve the methods for isolation and assessment of microvesicles from blood because these methods are poorly repeatable.”

The team will create a bank of frozen microvesicle isolates from blood of patients who have pancreatic cancer, colorectal cancer, or unexplained thromboembolism, as well as healthy subjects. They will then perform proteomic analysis on these populations. Patients with cancer will be followed for concentration and composition of microvesicles in blood isolates during their treatment. The effect of external parameters (blood flow through the needle in sampling, sedimentation in the centrifuge, volume of blood sampled, and temperature during the isolation process) on the contents of isolates will also be investigated, and theoretical models on the mediated interaction between membranes as a possible anticoagulant and antimetastatic mechanism will be considered.

“The work of the Slovenian Research Agency is a major improvement in the transparency of funding research compared to the way this was done in the previous totalitarian regime,” says Professor Kralj-Iglič. “However, as some influential persons still continue to hold important positions in science and politics, there are still attempts to retain privileged status for some and prevent independent researchers from obtaining funding, mostly by means of influencing the definition of criteria and rules.”

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Investigating the Predictive Value of Thrombin Generation Tests

Mojca Stegnar, PhD, head of the Clinical Lab, University Medical Centre, Department of Vascular Diseases, Ljubljana, Slovenia, until August 2010, now retired but continuing to conduct research in the department

Professor Stegnar received a 3-year grant from the Slovenian Research Agency in April 2009. The grant provides material expenses and a contribution towards salaries. Professor Stegnar explains, “The grant is being used to conduct research on the predictive value of thrombin generation tests for outcome after revascularisation procedures and for effectiveness of anticoagulant treatment.”

The project is being conducted in collaboration with the Department of Clinical Sciences, Danderyd Hospital, Karolinska Institute, Stockholm, Sweden. Together they

aim to establish whether lab tests for determination of thrombin generation (eg, calibrated automated thrombography or overall haemostatic potential) can be used to identify patients with increased risk for restenosis/reocclusion after vascular procedures such as percutaneous transluminal angioplasty, and whether these tests are superior to prothrombin time for monitoring haemostasis correction after oral anticoagulant treatment interruption in patients requiring invasive procedures.

In the past, Professor Stegnar studied haemostasis activation in thrombophilia¹



Professor Stegnar in the lab. Photograph courtesy of Professor Stegnar.

and the influence of sequence variations in factor VII, gamma-glutamyl carboxylase, and vitamin K epoxide reductase complex genes on warfarin dose requirement.² She has also shown that high levels of D-dimer levels predict cardiovascular events in patients with chronic atrial fibrillation during oral anticoagulant therapy.³

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Looking for Preclinical Markers of Atherosclerosis

Pavel Poredoš, MD, PhD, FESC, full professor of internal medicine, Medical Faculty, Ljubljana, and chief consultant and adviser of vascular medicine, Department for Vascular Disease, University Medical Centre of Ljubljana

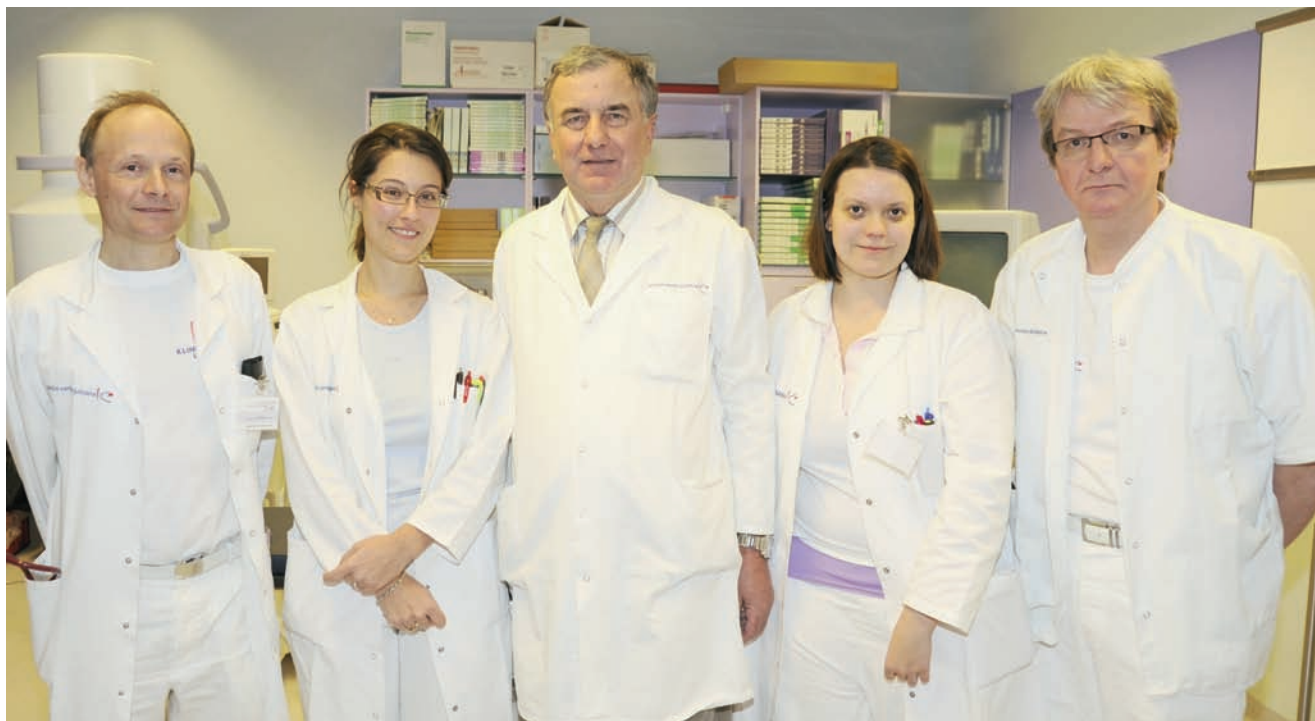
Professor Poredoš received a grant from the Slovenian Research Agency for his research group in the Department of Vascular Disease at the University Medical Centre of Ljubljana for the first time 15 years ago. He has renewed it every 4 years. The last renewal of the contract started in

2010 and will expire in 2015. This grant provides financial support for basic and clinical research. It covers the expenses for equipment, lab materials, and awards for researchers.

The research team is composed of 12 medical doctors, 10 of whom have PhDs and are employed full time at the centre as internal medicine specialists. With the funding, they have been investigating preclinical atherosclerosis, endothelial dysfunction, morphological deterioration of the arterial wall, and circulating markers of the atherosclerotic process. Using positron emission tomography, they study inflammatory activity in the atherosclerotic arterial wall in vivo and compare these findings to histological and histochemical results in specimens of carotid lesions collected during carotid endarterectomy. Furthermore, they use several methods to study the effect of different drugs on endothelial dysfunction in patients with polycystic ovary syndrome.

The group is searching for preclinical markers of atherosclerosis in patients who have risk factors of atherosclerosis, atherosclerotic disease, and deep venous thrombosis. They were one of the first to show increased intima media thickness and deteriorating endothelial function in smokers. They also showed that the functional and morphological deterioration of the peripheral arteries depends on the duration and number of smoked cigarettes.¹ They also found endothelial dysfunction related to microalbuminuria in children with insulin-dependent diabetes mellitus.²

Further investigations of endothelial function were performed in other vascular diseases and in nonatherosclerotic



Professor Poredoš and his research team at the University Clinical Center, Department of Vascular Disease, Ljubljana. From left to the right: Professor Aleš Blinc, MD, PhD, Assistant Professor Mateja Kaja Ježovnik, MD, PhD, Professor Poredoš, Urška Bregar, MD, Associate Professor Zlatko Fras, MD, PhD. Photograph courtesy of Professor Poredoš.

diseases such as Buerger's disease and systemic lupus erythematosus. They found that patients with Buerger's disease have significantly reduced endothelial-dependent, flow-mediated dilation capability of the brachial artery and that this decrease is related to circulating inflammatory markers.³

In patients with lupus erythematosus they showed that endothelium-dependent and -independent dilation capability of the peripheral arteries is decreased and that the presence of antiphospholipid syndrome intensifies this disturbance.⁴ Interventional studies investigated the influence of different drugs on endothelial function.

Recently, Professor Poredoš and his colleagues investigated the pathogenetic mechanism of venous thrombosis and found that idiopathic venous thrombosis is closely related to preclinical indicators of atherosclerosis.⁵ Patients with deep venous thrombosis, like subjects with risk factors of atherosclerosis and atherosclerotic disease, have significantly reduced endothelium-dependent flow-mediated dilation of the brachial artery, which could be involved in the pathogenesis of deep venous thrombosis.⁶ Increased systemic inflammatory markers were also demonstrated in patients with idiopathic deep venous thrombosis and were closely related to endothelial dysfunction.

Professor Poredoš says, "These results show that inflammation is probably a basic mechanism of the development of deep venous thrombosis and causes endothelial dysfunction and stimulates thrombogenesis."⁷

He adds, "We were also the first to show in patients with idiopathic VT [venous thrombosis] not only endothelium-dependent but also endothelium-independent dilation capability of the brachial artery is decreased and as such could be involved in the pathogenesis of VT."

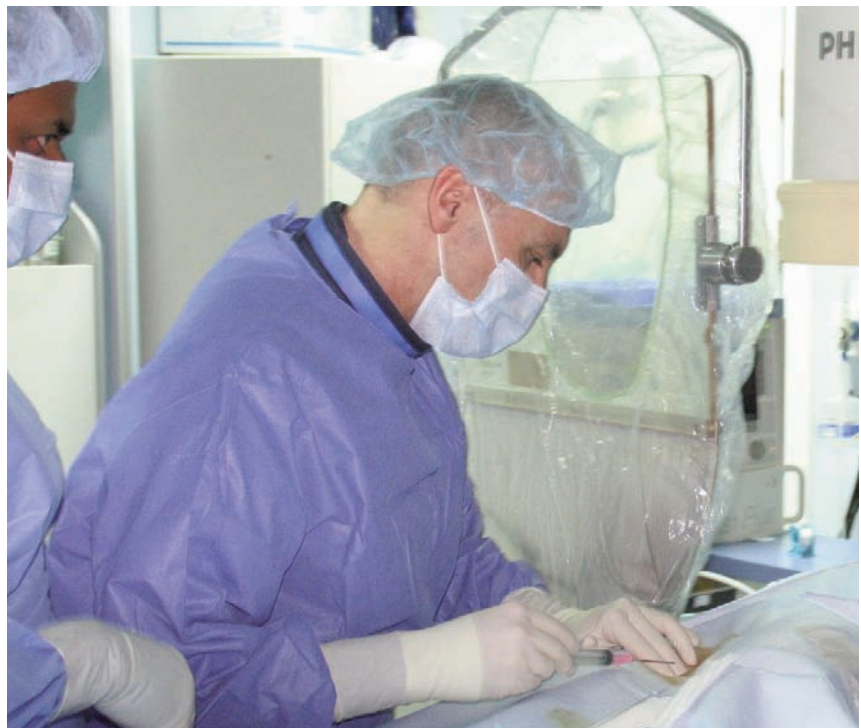
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Investigating How to Maximise the Effectiveness of a Primary Percutaneous Coronary Intervention Network

Professor Marko Noč, MD, PhD, FESC, professor of medicine, Medical School of Ljubljana, chief, Centre for Intensive Internal Medicine, and interventional cardiologist, University Medical Center, Ljubljana

Professor Noč received a programme grant from the Slovenian Research Agency to work on emergency situations in internal medicine. His work on primary percutaneous coronary intervention patients with ST-elevation myocardial infarction led to an initial grant for the period



Above, Professor Noč. Right, Professor Noč carrying out a primary percutaneous coronary intervention. Photographs courtesy of Professor Noč.

from 2004 to 2009. The success of the research led to the grant being extended from 2009 to 2014.

Professor Noč uses the grant to cover expenses for equipment and research materials and a minor contribution to the salaries of research group members (cardiologists and critical care physicians who work in the cardiac intensive care unit at the university teaching hospital).

The research is clinically oriented and covers acute coronary syndromes and sudden cardiac arrest. “We designed and proved the effectiveness of a primary percutaneous coronary intervention network for ST-elevation myocardial infarction and refined primary percutaneous coronary intervention with regard to concomitant pharmacotherapy and coronary thrombus aspiration,” says Professor Noč.

“During the past [few] years, we have focused on patients with resuscitated sudden cardiac arrest in whom we investigated potential coronary causes and the effectiveness of cardiac arrest percutaneous coronary intervention.

“We also investigated the feasibility, safety, and effectiveness of simultaneous urgent invasive coronary strategy and mild induced hypothermia in comatose survivors of cardiac arrest.”

Monitoring Coronary Atherosclerosis and Calcification in Chronic Kidney Disease

Breda Pečovnik Balon, MD, PhD, vice dean for international affairs, Medical Faculty, University Clinical Centre Maribor, University of Maribor, Maribor, Slovenia

Professor Pečovnik Balon received a 3-year grant in April 2009 to monitor atherosclerosis and calcification of coronary vessels in patients with chronic kidney disease and evaluate their contribution to renal failure. She is conducting

the research in collaboration with doctors from the Dialysis and Nephrology Departments at the University Clinical Center, University of Maribor.

Professor Pečovnik Balon has been researching chronic kidney disease and mineral and bone disorders for the past 15 years. Recently, Professor Balon and her coworkers showed that 48-hour ambulatory blood pressure measurement is an independent predictor of cardiovascular death in patients on haemodialysis,¹ and described correlations between left ventricular mass index and blood pressure in patients on haemodialysis.²

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Characterising and Cloning the Venom Components of the Nose-Horned Viper That Affect the Human Haemostatic System

Igor Križaj, PhD, scientific counsellor, group leader, and head of the Department of Molecular and Biomedical Sciences, Jožef Stefan Institute, Ljubljana, with appointments at the Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, and at the Centre of Excellence for Integrated Approaches in Chemistry and Biology of Proteins, Ljubljana

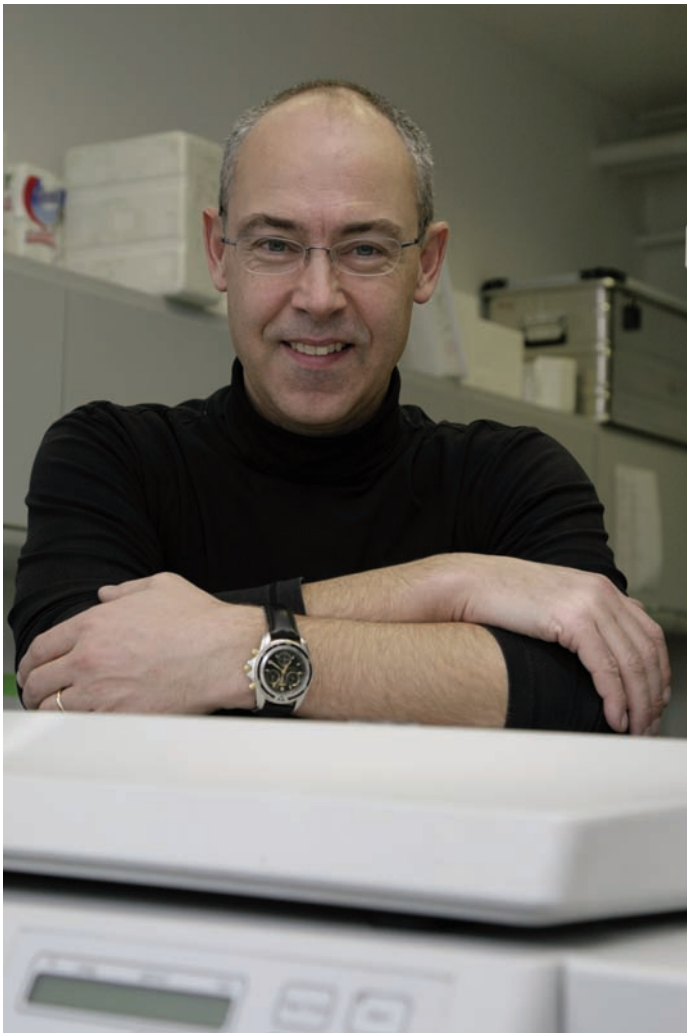
In July 2011, Professor Križaj received funding of 4.45 full-time equivalents over 3 years (total budget ≈€300 000) for the project titled “Discovering Innovative Drugs for



Professor Pečovnik Balon with her coworkers of the Nephrology and Dialysis Department, University Clinical Centre Maribor. From left to the right: Professor Pečovnik Balon, Assistant Professor Robert Ekart, MD, PhD, Benjamin Dvoršak, MD, Assistant Professor Sebastjan Bevc, MD, PhD, and Professor Radovan Hojs, MD, PhD. Photograph courtesy of Professor Pečovnik Balon.

Regulation of Haemostasis by Venomics of the *Vipera ammodytes ammodytes* Snake.” The grant is divided between 2 partner institutions: Jožef Stefan Institute, which is the leading institution (3.56 full-time equivalents), and the Division of Paediatrics at the University of Ljubljana Medical Centre (0.87 full-time equivalents). The project is also linked to an international bilateral project involving researchers from the Department for Research and Development, Institute of Immunology, Zagreb, Croatia.

The nose-horned viper *Vipera ammodytes ammodytes* has an arsenal of proteins targeting a variety of processes in the human haemostatic system.¹ Professor Križaj says, “The goal of the project is to understand and exploit the venom machinery that produces these effects to discover novel diagnostic and therapeutic procedures for curing human haemostatic disorders.”



Professor Križaj in the lab. Photograph courtesy of Professor Križaj.

Using a proteomic approach, the team will pharmacologically, structurally, and biochemically characterise the venom components that affect the human haemostatic system, thereby constructing a natural library. Venom components with the highest potential for improving or creating therapies and diagnostic procedures in clinics will be cloned for further in-depth characterisation and comprise a synthetic library.

The team is currently developing 2 venom components, ammodytase² and ammodytotoxin,³ in which pharmacological activity has already been recognised as medically interesting. Ammodytase is a fibrinolytic metalloproteinase that has been further developed as a new type of antithrombotic compound. Ammodytotoxin is a phospholipase A₂ with a potent anticoagulant action due to its high-affinity binding to activated blood coagulation factor X.⁴ “We are designing short peptides or peptidomimetics on the basis of the ammodytotoxin structure⁵ interacting with activated blood coagulation factor X that could be used to prevent blood coagulation,” says Professor Križaj.

The first investigations of snake venom components affecting the human haemostatic system by Professor Križaj and his colleagues were made about a decade ago. Discovery of the anticoagulant activity of ammodytotoxin resulted in 3 consecutive international bilateral projects—called the Proteus Project—with a partner group from the Pasteur Institute in Paris, France.

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