RCH FOLLOWING HUMAN ORGAN TRANSPLANTATION AND DIFFERENTIAL SCANNING CALORIMETRY ANALYSIS OF HUMAN PLASMA IN MELANOMA MALIGNUM

Ph.D. thesis

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BREVIATIONS

AU:	Arbitrary Unit
BCC:	BasoCellular Carcinoma
BU:	Bergmayer Unit
CAN:	Chronic Allograft Nephropathy
CN:	Carbamid Nitrogen
CNIs:	CalciNeurin Inhibitors
DN:	Dysplastic Naevi
DSC:	Differential Scanning Calorimetry
EBPG:	European Best Practice Guidelines
GSH:	Reduced glutathione
HPV:	Human Papilloma Virus
IU:	International Unit
KAT:	Catalase
LDH:	Lactate DeHydrogenase
LIF:	Leukemia Inhibitory Factor
M-CSF:	Macrophage Colony Stimulating Factor
MDA:	MalonDiAldehyde
MM:	Melanoma Malignum
MPO:	Myeloperoxidase
MMF:	Mycophenolat Mofetil
mTOR:	Mammalian Target Of Rapamycin
NK cells:	Natural Killer cells
nmol/ml	Nanomol/milliliter
NMSC:	Non-Melanoma Skin Cancer
OFRs:	Oxygen Free Radicals
PIBF:	Progesterone Induced Blocking Factor
PMN:	PolyMorphoNuclear
S6K:	S6 kinase
SCC:	SquamoCellular Carcinoma
SOD:	SuperOxide Dismutase
SRL:	Sirolimus

rowth Factor-ƃ

UV irradiation: UltraViolent irradiation

VEGF: Vascular Endothelial Growth Factor

1/1 INTRODUCTION

Non-melanoma skin cancer (NMSC) is the most common malignancy among organ transplant recipients. Among the types of NMSC, the incidence of basal cell carcinoma (BCC) is 10-fold higher than in the general population, while the squamous cell carcinoma (SCC) has a 100-times greater risk than the general population. Moreover, the usual BCC/SCC incidence ratio of 4:1 is being reversed in transplant recipients. Several large studies have reported that risk factors include the use of immunosuppressive drugs, sunlight exposure, genetic background, viral infection, race and older age. The European Best Practice Guidelines (EBPG) has been published in 2002, which summarized the importance of prevention and treatment of skin cancer in transplant organ recipients.

1/2 AIMS AND HYPOTHESIS

The aim of the present study was to set the first Hungarian dermatological screening program to establish the incidence and clinical characteristics of NMSC after organ transplantation.

1/3 PATIENTS AND METHODS

116 adult, white skin-typed kidney or simultaneous pancreas-kidney transplanted patients (70 male, 46 female; median age: 49.3 years) have been involved from September of 2008 on the Surgical Clinic of Pécs University. All patients were examined for NMSC by a full skin examination, and they filled a standardized questionnaire. Data were collected on cumulative occupational and recreational sun exposure, skin type, history of 2 or more painful sunburns, suncream usage, personal or family history of skin cancer, time from transplantation, immunosuppression protocol, type and localization of NMSC.

Statistical analysis. Results are expressed as mean ± SD. Statistical analysis was performed using Studentõs t-test. The level of significance was set at $p < 0.05$.

sulted in 16 NMSC (13.8 %, male/female=1:1) at 11 patients. The median age in the NMSC group was higher than in the non-cancer patients (60.6±1 vs. 45.2±2 years, $p < 0.05$). Histological examination resulted in 13 BBC (81.25 %) and 3 SCC (19.75 %). The ratio of BBC/SCC was 4:1. Localization of NMSC was significantly higher on the head and the neck (13 vs. 16; 81.25 %; $p < 0.05$), than on the upper limbs (2 vs. 16; 12.5 %) or on the trunk (1 vs. 16; 6.25 %). A median duration time since transplantation was 4.1 years.

Incidence of NMSC was significantly higher at patients, who had outdoor workplace or more than 2 painful sunburns prior to transplantation (10 vs. 11; 90.9 %; $p < 0.05$). Only 1 patient used sunscreen in the NMSC group, and its usage was low also in the non-cancer patients (23 vs. 105; 22 %). Ten recipients used cyclosporine as immunosuppressant from the 11 NMSC patients (10 vs. 11, 90.9 %; $p < 0.05$), and it was 43 (43 vs. 105, 41%) who had no skin cancer. Mycophenolate mofetil treatment was approximately equal in groups with (82 %) or without NMSC (92 %). Eighty percent of transplanted patients had never been on a dermatological skin cancer screening examination after transplantation.

1/5 DISCUSSION

All currently available immunosuppressive agents cause non-specific immunosuppression and hence increase the risk of infection and malignancy. Skin cancers are the most common post-transplant malignancies after organ transplantation.

According to the guideline of EBPG, we started dermatological skin cancer screening program resulted 13.8 % NMSC in our center. Histological examination showed BCC in 13 and SCC in 3 cases. Thus, the BCC/SCC ratio was 4:1 in our experience, which correlates to the ratio found in the general population. However, numerous studies reported that reverse ratio is found in organ transplant recipients. According to our data the ratio is not reversed, but its reason is not known for us. To clarify these results more patients have to be involved in this program from our region. One interesting piece of data exists in the literature, namely, that the ratio was 2:1 and was maintained near the general population after long term calcineurin inhibitor monotherapy. It reversed only following bi- or tritherapy usage.

experience was significantly higher on the head or on the trunk. These data confirmed that the localization of skin cancers essentially in areas exposed to light is a strong indication that the sun is a factor in the development of these tumours. A median duration time since transplantation was 4.1 years. These data correlated to the international results. However, only the regular screening examination should give us the correct length of time between the transplantation and the presence of ñde novoò skin cancers.

Epidermatological and molecular data strongly suggest that NMSC are associated with excessive exposure to the ultraviolet (UV) radiation in sunlight. A particularly important factor is UV light after organ transplantation. According to our results, in patients who had 2 or more painful sunburns during their life or who had excessive sunlight exposure (outdoor workplace and/or recreation) significantly more NMSC were found. At present, 30 % of our patients who are without NMSC had 2 ore more sunburns during their life, which indicates the importance of the information and the preventive effect of the regular screening program for the exposed patients. In our patients the conscious protection against harmful sunlight with use of sunscreen or protective clothing was extremely low. In these high-risk patients more frequent control (at least at yearly intervals), their conscious avoidance of sun exposure and compliance are necessary for the primary prevention.

Another important factor in the development of post-transplant skin cancers is the immunosuppressant treatment. Immunosuppressive drugs may accelerate the development of NMSC in transplanted patients through 2 distinct mechanisms. First, the agents used may be directly carcinogenic. Second, the chronic immunosuppression creates a state in which immune surveillance and eradication of precancerous changes are impaired. Numerous prospective studies confirmed that calcineurin inhibitors have direct carcinogenic effect. In our experience the incidence was significantly higher at patients who had been treated with cyclosporine. The main reason for this is that cyclosporine increases the production of growth factors during tumour progression. According to the data in the literature the mycophenolate mofetil (MMF) has an antiproliferative effect. So, skin cancer and other solid tumours developed rarely during MMF administration. At the background of our results should stand the tumour-protective effect of MMF.

In summary NMSC, the most common post-transplantation malignancy has

ity and mortality. The dermatological screening
splantation center. It is a simple and free from inconvenience, and patients received it with a positive attitude. So, intensive education, primary prevention, early intervention and close follow-up are the key components of the program to lessen the burden of cutaneous complications in these patients, which we will continue in the future. The therapeutic management of transplanted patients with skin cancer is best done in a multidisciplinary fashion, integrating dermatologists, dermatologic and transplant surgeons.

2/1 INTRODUCTION

Transplanted patients are at high risk of developing malignancy, most frequently NMSC, which is largely attributed to decreased tumour surveillance arising from immunosuppression, in conjunction with ultraviolet (UV) radiation exposure. From NMSC, the basal cell carcinoma (BCC) occurs with 10-times, while the squamous cell carcinoma (SCC) at 100-times higher risk than in the general population. Furthermore, UV radiation can also generate oxygen free radicals (OFRs) leading to oxidative stress and oxidative modification of various molecules, including DNA, proteins and lipids

2/2 AIMS AND HYPOTHESIS.

The aim of the present study was to examine the changes of oxidative stress parameters on transplanted patients with or without NMSC.

2/3 PATIENTS AND METHODS

According to the European Best Practice Guidelines (EBPG) our team started the first Hungarian dermatological screening program, where 116 adult, white skin-typed kidney or simultaneous pancreas-kidney transplanted patients (70 male, 46 female; median age: 49.3 years) have been involved on the Surgical Clinic of Pécs University. Dermatology follow-up resulted from 16 NMSC (13.8%) 13 BBC and 3 SCC. Within the screening program to monitor the oxidative stress parameters peripheral blood samples were collected from each patient.

Biochemical Assays

The concentrations of malondialdehyde (MDA) and reduced glutathione (GSH) in blood samples were measured by a lipid peroxidation assay kit and a glutathione assay kit (Calbiochem) according to the manufacturerõs instructions. Final values were given as nanomoles per millilitre. The superoxide dismutase (SOD) activity was assessed using the superoxide dismutase assay kit (Calbiochem) according to the manufacturerõs protocol. Values of SOD activity were expressed in international units

ps 100 µl plasma, 100 µl Ellmanôs reagent (1 mM EDTA containing TRIS buffer were mixed and photometry was performed at 412 nm. Its amount was expressed in nanomoles per millilitre. The production of OFRs was determined in whole blood with chemiluminescense method based upon the reaction of luminol with free radicals. To sum up, 20 εl EDTA anticoagulated blood was diluted in 1400 µl Dulbeccoôs modified Eagleôs medium nutrient mixture of 37ºC. 30 εl of 3-aminophtalhidrazide was added and the cuvette was immediately placed to Chrono-Log Whole Blood Lumi-aggregometer. After determining the spontaneous radical production, 50 εl phorbol-12 myristate-13 acetate (PMA) was injected into the cuvette and the resulting light output was recorded on a chart recorder. The peak value of free radical production was calculated from the recorded curve, and the results were related to the white blood cell counts. The normal peak value is below 10.9±2.6 AU. Plasma myeloperixidase (MPO) level was obtained by adding 200 εl of plasma to 1ml mixed solution (10.9 ml Na citrate, 100 εl o-Dianisidin, 1ml H_2O_2 and 5 εl of 0.05% Triton-x-100). Incubation followed at 37ºC for 5 minutes. After adding 1 ml of 35% perchloric acid to the solution, it was centrifuged for 10 minutes at 2500 rpm and was measured at 560 nm. The normal value is below 0.41±0.10 Bergmayer units per millilitre (BU/ml). Catalase (Cat) enzyme metabolizes H_2O_2 to water and oxygen preventing the production of secondary generation of toxic intermediates. Catalase enzyme activity in whole blood was determined by the method of Aebi. Value of control population is 1931±72 Bergmayer units per millilitre.

Statistical analysis. Results are expressed as mean ± SD. Statistical analysis was performed using Studentôs t-test. The level of significance was set at $p < 0.05$.

he plasma MDA concentration was significantly elevated compared to healthy controls ($p < 0.05$), while the MDA in haemolysate increased slightly. In peripheral blood samples both the plasma MDA and haemolysate MDA values increased significantly in the NMSC group compared to the healthy control group and to the group without NMSC ($p < 0.05$). The levels of GSH and -SH groups were significantly decreased compared to healthy controls. Among the antioxidant enzymes, the activity of MPO and Cat elevated significantly in the group without NMSC. Furthermore, their activities were significantly higher in the NMSC group. SOD activity decreased slightly, but not significantly. Total production of OFRs was significantly higher in the NMSC group than in the group without NMSC or than in the healthy controls ($p < 0.05$).

2/5 DISCUSSION

Although drug-induced immunosuppression is an obvious factor in the increased susceptibility of transplanted patents to skin cancer, compared to non-transplanted population, this is unlikely to explain the differences within the transplant group. Increasing evidence suggests that oxidative stress induced by UV radiation and immunosuppression together may predispose certain transplanted patients to NMSC.

As part of the screening program we measured the oxidative stress parameters from peripheral blood samples. Our results showed that lipidperoxidation and total production of OFRs was significantly higher in patients in the NMSC group compared to healthy controls and to the group without NMSC. Increasing evidence suggests that elevated lipidperoxidation is not the reason but the consequence of the UV induced photocarcinogenesis. UV radiation increases the activity of xanthine oxidase in human keratinocytes, increasing production of OFRs, which can cause further damage in DNA, in proteins and lipids. Lipidperoxidation induces inflammation with prostaglandin production in the skin, which is presented at clinically as solar keratosis (SK). A change in the SK may trigger the inflammatory response, which in turn drives the beginning SK into developing into NMSC, mainly to SCC. Moreover, the high risk of transplanted patients comes from the fact that they receive the UV-induced immunosuppression locally and they are treated with immunosuppression drugs systematically.

o demonstrated that the levels of GSH and -SH compared to healthy controls. Moreover, from the antioxidant enzymes, the activity of MPO and Cat elevated significantly in groups with or without NMSC. Furthermore, their activities were significantly higher in the NMSC group. These results indicate the decreased endogenous scavanger capacity and the elevated antioxidant capacity against the increased production of OFRs. Several studies showed that increased production of OFRs following exposure to UV can deplete these antioxidant defences, leaving the skin vulnerable to attack from OFRs.

3/1 INTRODUCTION

Graft rejection is a dangerous adverse event after renal transplantation. Despite the impressive reduction in early acute rejection rates over the past decades, chronic allograft dysfunction remains a key issue after transplantation. Chronic rejection, the primary cause of late renal allograft loss, results from a complex interplay between immunological and non-immunological factors. Natural killer (NK) cells, play an important role in this processes by regulating T and B cell-mediated rejection through secretion of cytokines.

NK cells also play a role in the feto-maternal relationship. High peripheral NK activity was shown in recurrent miscarriage and implantation failure. Progesterone is critical for the establishment and the maintenance of pregnancy, both by its endocrine and immunological effects. The immunological effects of progesterone are mediated by a protein named Progesterone-Induced Blocking Factor (PIBF). PIBF is indispensable for to the success of pregnancy in mice, and that the major part of its pregnancy-protective effect is due to its NK cell inhibitory activity. In humans, the concentration of PIBF in the urine and in the serum is related to the outcome of pregnancy. Rejection of transplanted allograft and pregnancy loss share the same evolutionary origin.

3/2 AIMS AND HYPOTHESIS

The objective of the present study was to examine changes PIBF concentration in urine of transplanted patients with or without graft rejection.

3/3 PATIENTS AND METHODS

This study included 116 patients (70 men and 46 women; median age 49.3 years) who had undergone kidney transplantation. The median duration since transplantation was 3.46 years. The average interval between the onset of renal disease and sampling was 12.3 years, while the median interval between graft rejection and our study was 1.75 years. Patients were divided in equal numbers per group, according the following criteria: rejection episodes in the history (with ï or

reatinine (CN) and blood urea nitrogen (BUN) values). Routine blood tests (CN, BUN) were performed in Department of Laboratory Medicine of Pécs University. CN concentrations were considered normal between 180 and 350 micromoles per liter; concentration of BUN between 2 to 9 millimoles per liter. Urine samples were collected from all selected patients to measure PIBF concentration.

ELISA test for PIBF. Urine samples were used during overnight incubation at 4°C, 96-well microtiter plates were coated either with anti-human 48-kDa recombinant PIBF IgG in 50 mM carbonate buffer (plate 1), or with human 48-kDa recombinant PIBF in 0.5 M Tris buffer (plate 2). For generating a standard curve, recombinant buffer was incubated with a standard amount of biotin-labelled antirecombinant PIBF IgG (400 ng) for 60 min at 37°C. Urine samples were diluted 1:2.5 and 1:5 and incubated with 400 ng biotin-labelled antirecombinant PIBF IgG in 0.5 M PBS for 60 min at 37°C before being added to ELISA plate 1. During the 1 h incubation nonspecific binding sites on plate 2 (coated with human recombinant PIBF) were blocked with 200 ml of 0.1% BSA, 0.5% gelatine in PBS-Tween. After this incubation step, 100 ml standard solutions or the urine samples were transferred from plate 1 to plate 2 and incubated for 1 h. After washing the plates three times with PBS Tween, 100 ml of 1:1000 diluted horse radish peroxidase)-conjugated Streptavidine (AP Hungary Ltd) in 0.1% BSA PBS-Tween was added. The reaction was developed by adding the substrate OPD (orthophenylene-diamine; Sigma, Hungary) and measured at 495 nm. Final values are given as nanogram per milliliter.

Statistical analysis was performed using the *t* test. Results are given as mean (SD). $p < 0.05$ was considered significant.

rine decreased significantly in patients who had one or more rejections compared with patients without rejection episodes in the history (22.7±1.2 ng/ml vs, 31.8±2.2 ng/ml)) ($p < 0.01$). This decrease was independent from the number of rejections, and from patientsôgender. Moreover, the PIBF concentrations in urine were significantly lower (27.2±1.4 ng/ml) in patients with increased creatinin levels compare to those with normal CN values (38.1±2 ng/ml) ($p < 0.05$). PIBF concentrations were also related to high and low BUN concentrations (20.4±1.5 ng/ml and 33.1±2.2 ng/ml respectively; $p < 0.01$)

3/5 DISCUSSION

In spite of more potent immunosuppressive strategies late failure of transplanted kidney continues to be a major problem in organ recipients. The gold standards for the diagnosis of graft dysfunction and rejection include renal blood tests (creatinine, BUN) and allograft biopsy evaluated with conventional histology. However, prevention of CAN and identifying early markers of disease progression is currently one of the main goals for improvement of kidney graft survival rates.

The present study investigated the changes of PIBF concentration in urine samples after human renal transplantation. Urine PIBF value were significantly lower in patients who had one or more rejection compared with patients without any rejection episodes in the history. The exact relationship between PIBF level and previous graft rejection in the present study is not yet known. Several studies show, however, that PIBF blocks peripheral blood NK cell activity and by this plays a role in the maintenance of pregnancy. By manipulating the intracellular concentration of PIBF in vitro, one can modulate the killing activity of human peripheral blood NK cells. Neutralization of endogenous PIBF activity in pregnant mice by anti-PIBF antibody results in a 70% reduction in the number of viable foetuses, and this is associated with an increased splenic NK activity. Furthermore, numerous publications have shown the importance of NK cells in exacerbation of T-cell responses to predict the risk of rejection following organ transplantation. In accordance with the observations that NK cells play a central role both in organ rejection and foetal loss, changes in PIBF concentrations seem to be susceptible to indicate renal rejection episodes in patients.

In the present study, we also demonstrated that PIBF level of urine was significantly lower in that patients, who had increased creatinin and urea nitrogen in

ation reflected the acute renal graft failure, which rejection episodes. Direct explanation on the background of this result is not yet known. However, the urine seems an obvious choice for evaluating immune activity in the organ of its synthesis. Only a few reports have focused on urine as a marker of graft function or immune reactivity of patients. Moreover, previous reports in pregnant animal model demonstrated that one of the main mechanisms by which NK cells kill their target cells is the exocytosis of perforin containing granules. In the presence of PIBF, activated NK cells fail to release perforin from storage granules, and as a result, they do not lyse target cells.

orimetry analysis of human plasma in melanoma patients

4/1. INTRODUCTION

Cutaneus melanoma is the most malignant tumour of the skin and its incidence is on the rise. There is convincing evidence from epidemiologic studies that endogenous (genetic markers, skin type) and exogenous (UV irradiation) risk factors are in the development of melanoma malignum (MM). Early detection of primary melanoma assures increased survival, advanced MM has a poor prognosis and survival. The treatment primarily includes surgical removal of the tumour and adjuvant therapy (chemo-, immuno- and radiation therapy). The clinicopathological stage of the melanoma patients can determine by pathological evaluation of the primary lesion and of the dissected lymph nodes, as well as by routine examinations.

In 1969, Clark et al. proposed staging criteria for lesions on the basis of skin invasion levels. Subsequently, Breslow evidenced the importance of the primary melanoma thickness in millimeters, and this index became one of the most important prognostic indicators, in association with data on ulceration, mitosis and regression. Moreover, regional lymph node (sentinel) status has emerged as an accurate method for evaluating the draining lymph node basin, allowing for the generation of valuable prognostic information. Recently, there is a need for more studies and methods to monitor in MM patients in any stages.

Differential Scanning Calorimetry (DSC) is unsurpassed for understanding the stability of biological systems. DSC directly measures the stability and unfolding of a protein, lipid, or nucleic acid that occur in bio-molecules during controlled increase or decrease in temperature, making it possible to study materials in their native state. The DSC thermogram is an unique signature for a bio-molecules reflecting the normal or pathomorphological changes under given solution conditions. Therefore, DSC technique allows demonstrating the thermal consequences of conformation changes in different bio-molecules not only in the animal experiences, but in several surgical and oncological clinical studies.

developing the application of DSC approach as a new diagnostic and monitoring method for MM patients with or without regional lymph node and distant metastases.

4/3 PATIENTS AND METHODS

The study included 36 white adults (25 men and 11 women; median age 63.7 years). From routine histopathological parameters tumour thickness were evaluated according to Breslow which parameter changed from 0.5 mm to 8.3 mm in our patients. Invasion value was between Clark level II and IV in this study. Regional lymphatic infiltration was evaluated as a prognostic factor, and the sentinel lymph node was positive in 8 cases. The study included 5 patients with distant metastases (lung, liver).

DSC measurements

The thermal unfolding of the human plasma components were monitored by SETARAM Micro DSCII calorimeter. All experiments were conducted between 0 and 100°C. The heating rate was 0.3 K/min in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 εL sample volume in average. Reference sample was contained normal saline (0.9% NaCl). The sample and reference samples were equilibrated with a precision of ± 0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference samples. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration.

4/4 RESULTS

Our results showed that comparison of DSC scan of healthy controls with the curves of cases with melanoma, regional and distant metastases. The DSC measurements showed at least two marked different thermal domains during the denaturation. The first T_ms were only slightly influenced by the Breslow's depth and the Clark level, but it can be seen a difference in the melting enthalpies. The second T_ms and the calorimetric enthalpy changes demonstrated a significant difference of

in 0.95-8 mm range and in Clark levels of II-IV. een changed significantly in comparing with the control samples which were: T_ms 56 and 63 ºC, ΔH~1.5 J/g. In the pathologic samples and in the progress of the decease one can separate a third thermal component between the first and second T_m. It is at around 62 ºC, and it is shifted to higher temperature in case of regional metastasis and distant metastasis. To identify the proper plasma compounds we have to perform their separation with electrophoresis.

4/5 DISCUSSION

Malignant melanoma is one of the most aggressive malignancies in human and is responsible for almost 60% of lethal skin tumours. Its incidence has been increasing in white population in the past two decades.

Adequate resection of the specimens and sentinel lymph node biopsies are important factors in management of MM. But, there is no definite proof that longevity of patients is affected by routine laboratory tests. The human plasma proteome holds great promise as a convenient specimen for disease diagnosis and therapeutic monitoring. Moreover, blood samples may be easily obtained from patients by minimally invasive, safe procedure. The novel calorimetric assays are described that provides a new window through which to view the properties of the human plasma proteome. This study investigated the thermal changes of human blood plasma components in melanoma patients with or without regional lymph node and distant metastases by DSC. Overview 36 patientsô thermograms, we observed their individual characteristics compare to healthy controls.

In the present study, DSC scan of healthy controls and the curves of MM cases showed 2 different thermal domains during the denaturation. Examination of DSC data in different clinical stages of MM patients should observed closed correlation with melanoma thickness and the extent of regional invasion and distant metastases. These facts are important for many reasons: DSC measurement is suitable not only to clear skin cancer diagnosis, but to separate the different stages of MM patients and to monitor the actual stage of individualôs disease. However, there are no data in the literature indicating the possible diagnostic and staging method of human blood plasma by DSC in MM patients. The exact explanations of these results are not yet known. However, DSC analysis of plasma from diseased individuals

thermogram which are suggested to result not of the major plasma proteins but from interactions of small molecules or peptides with these proteins.

ÚJ MEGFIGYELÉSEK – NOVEL FINDINGS

1. According to the guideline of EBPG, we started dermatological skin cancer screening program in our transplantation center. Furthermore we foremost attract attention in Hungary that therapeutic management of transplanted patients with skin cancer is best done in a multidisciplinary fashion, integrating dermatologists, dermatologic and transplant surgeons

2. Available evidence indicates that UV-induced oxidative stress is important in skin cancer development, mainly in transplanted patients. Our research indicates that an imbalance exists between pro- and antioxidant status in transplanted patients. According to examined parameters significant differences were found in patients with or without NMSC. Because of the relevant role that oxidative stress may play also during end-stage renal disease, further prospective studies are needed to confirm our results.

3. Our study investigated foremost the changes of PIBF concentration in urine samples after human renal transplantation. Urine PIBF value were significantly lower in patients who had one or more rejection compared with patients without any rejection episodes in the history. Available evidence indicates that PIBF value of urine might be a marker of renal graft failure and a predictive factor in graft rejection; however, further prospective studies are needed to confirm this concept

4. This is the first report examined thermal changes by DSC on human blood plasma in MM patients with different clinical stages. Blood collection is a simple procedure and convenient to perform, and the DSC thermogram confirmed unique signature for human plasma components reflecting the normal, the pathomorphological changes and staging differences in melanoma patients. Further studies are needed to elucidate these relationships, but this preliminary study indicates great potential for the application of DSC as a clinical diagnostic tool, for example during disease grading and staging processes.

NS AND PRESENTATIONS

Articles in connection with Thesis

1. **Fekecs T**, Kádár Zs, Battyáni Z, Kalmár-Nagy K, Szakály P, Wéber G, Horváth ŕ P, Ferencz A. A szervtranszplantációt követŐ non-melanoma bŐrtumourok klinikai vizsgálata.
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6. **Fekecs T**, Ferencz A, LŗŢrinczy D. DSC analysis of human plasma in melanoma patients with or without regional limph node metastases. (poster)
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   Magy Seb 2011; 64(4): 207ï 212 DOI: 10.1556/MaSeb.64.2011.4.5

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Data of the publication activity of the author

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