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NEW TRENDS IN CLINICAL BIOCHEMISTRY OF TRANSPLANTATION

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POSTERS

HYPERINSULINEMIA CHANGES IN THE HEPATIC RECOVERY AFTER LIVER TRANSPLANTATION: LINK WITH THE GROWTH HORMONE/INSULINE-LIKE GROWTH FACTOR 1 AXIS. A PRELIMINARY PROSPECTIVE STUDY

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Background/Aim: GH/IGF-1 axis abnormalities (high GH and low IGF-1 serum levels) contribute to hyperinsulinemia and insulin resistance of cirrhosis.
However, the influence of liver transplantation (OLTx) on cirrhotic hyperinsulinemia and the contribution of the perioperative GH/IGF-1 axis changes to insulin metabolism are unknown.
Methods: Plasma GH, IGF-1 and Insulin levels were measured sequentially at the start of operation, during OLT (at 30 minutes and 2 hours from reperfusion), at 24 hours from reperfusion, and in the morning on postoperative days (POD) 7, 30, and 90, in 15 cirrhotic patients undergoing OLT. Twenty age-matched healthy volunteers with normal liver function served as controls.
Results: The study group had significantly higher Insulin and GH levels and lower IGF-1 levels in the preoperative period compared with the controls. All patients achieved a complete functional hepatic recovery already 1 month after OLTx. Insulin levels peaked 24 hours from reperfusion and then decreased slowly until POD 90 without achieving normality range. On the contrary, GH and IGF-1 levels achieved near normal range within 1 week after OLT. A positive correlation resulted between GH levels measured 24 hours and 1 week after OLT and Insulin levels on POD 7, while we have not found any correlation between IGF-1 and Insulin levels.
Conclusions: An hyperinsulinemic peak in the first 24 postoperative hours of OLTx and a slow Insulin-level decrease in the first 3 postoperative months suggest the possible influence of immunosuppression drugs on Insulin resistance. On the contrary, the severe GH/IGF-1 axis impairment found in patients with end-stage cirrhosis reverted in the first hours after successful OLT and seemed to have a limited influence on recovery of Insulin metabolism.

ITALIAN UNIVERSITY STUDENTS' OPINION ON XENOTRANSPLANTATION

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The transplantation of animal organs into humans is currently at an experimental stages on animal models and many problems still have to be overcome in the biomedical, immunological and ethical field. Organ transplant from genetically modified animals could make up the shortfall in human organs providing a potentially unlimited number of organs and overcoming risks associated with the wait for transplantation. Little is known about the influence of schooling and the choice of university faculty on attitudes to xenotransplantation. The aims of this study were: 1. to evaluate university students' understanding of and attitudes to xenotransplantation; 2. to investigate socio-demographic, religious and educational determinants behind students' opinions on xenotransplantation.

Methods: A 19-item questionnaire was distributed to University students on 5 different courses in Padua, Italy. Statistical analysis: Chi-squared, Pearson's test; p-values <0.05 were considered significant.

Results: 585 out of 612 (95.6%) students completed the questionnaire (132 males, 453 females, mean age 20.4, range 19-23 years), from Medicine (33.85%), Educational Sciences (30.26%), Psychology (18.46%), Veterinary Medicine (11.45%) and Agriculture (5.98%). As concerns their previous schooling, they came from classic or scientific high school (58.3%), technical college (14.7%), teacher training college (11.9%), language college (6.3%), or from other schools (8.8%). As for their religious belief, 83.2% were Catholics (52.6% practicing), 11.6% indicated not to believe in any religion.

88% of the students knew of the possibility of transplanting animal organs into humans and 78% of them approved of this idea. When grouped according to gender, education and University course, a higher proportion of students who approved of xenotransplantation were male ($p=0.017$), had attended classical or scientific high school ($p=0.011$) and were on science course than those on art course ($p=0.013$). Disapproval for moral, ethical or religious reasons was higher among practising Catholics than among the non-practising Catholics, who rejected xenotransplantation for immunological and infectious reasons mainly ($p<0.02$).

Students on science courses would accept any organs ($p = 0.003$) from any animal donor ($p = 0.003$) in a significantly higher proportion than students on art courses. 47.5% of students didn't know of the genetic manipulation of animal organs before being transplanted in humans; males were more informed than females ($p<0.001$).

Conclusions: University students generally approved of xenotransplantation. Male gender, high school education and choice of faculty of science at University were associated with a greater acceptance of xenotransplantation. Practising vs. non-practising Catholics reported significantly different reasons for any disapproval of xenotransplantation.

BRAIN NATRIURETIC PEPTIDE (BNP) AND NT FRAGMENT AS LABORATORY BIOCHEMICAL MARKERS OF REHABILITATION FOR HEART TRANSPLANTATION IN CHRONIC HEART FAILURE PATIENTS

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Introduction: Nowadays the most appropriate type and scheduled program of physical exercise rehabilitation for Chronic Heart Failure (CHF) patients has not yet been identified[1]. Recently, some evidence suggested that local muscle training could help patients affected by CHF by improving aerobic working capacity[1]. In addition it was clearly shown that patients belonging to NYHA II and III classes gained advantages from physical exercise without deterioration of cardiac function [2]. According to recent data, the exercise intensity before heart transplantation can't benefit existing laboratory parameter so patient symptoms are currently the best parameter to define exercise strength graduation[3]. It is suggested that ANP (Atrial Natriuretic Peptide) and its fragments are produced by the atria and ventricles in early phases of CHF, and BNP (Brain Natriuretic Peptide) and its fragments later on. In particular proANP(1-98), proposed as a prognostic marker of CHF, LANH, an ANP peptide fragment particularly involved in physical exercise, and NT-proBNP, a good marker of left ventricular dysfunction, were evaluated before and after a training session. The aim of the study were in particular to evaluate how the rehabilitation program improves CHF patients clinical picture and how long a rehabilitation program should be prolonged to get a real remodeling of heart wall.

Methods: Nt-proANP, LANH and NT-proBNP together with the traditional parameters used to verify the effectiveness of a specific endurance rehabilitation program, such as echocardiographic parameters, maximum oxygen consumption (fast ramp test) and structured questionnaires on life quality and symptoms of heart failure (MSHFQ, RESTQ) and on all-day and sporting activity were measured and carried out in 20 CHF patients (NYHA I-II). They were randomly divided in two groups called A and B: 10 (group A) (68.4 ± 1.9 years; 1.72±0.02 m; 82.3±6.1 kg), performed a specific rehabilitation program (8 sets of exercises for different muscle groups, using training devices, at 65% of repetition maximum, with 2 x 12 repetitions per muscle group, 3 times per week for 12 weeks); 10 (group B) (70.9±2.1 years; 1.72±0.02 m; 79.4±3.1 kg), performed a low intensity endurance exercise (phase III cardiac rehabilitation program), including once a week supervised heart group training for 1 hour and self guided endurance training 3 times per week. Plasma samples from blood EDTA samples, collected after overnight fasting, were stored at -80° C until assay. Competitive EIA methods and the biotinilated analogues, the peroxidase streptavidin reaction and the detection at 450 nm were employed for Natriuretic Peptide assays[5]. Statistics: Friedman ANOVA (significance: $p<0.05$).

Results: Both proANP(1-98)(14.75± 1.59 vs 14.83 ± 1.90 nmol/L, respectively), LANH (2.04 ± 0.22 vs 1.93 ± 0.22 nmol/L) and NT-proBNP (806 ± 115 vs 710 ± 124 pmol/L) values did not vary comparing before with after rehabilitation programs in group A. The same results were observed in group B: proANP(1-98)(13.54 ± 4.44vs 13.35 ± 2.35 nmol/L, respectively), LANH (1.58 ± 0.33vs 1.40 ± 0.27nmol/L) and NT-proBNP (697 ± 128vs 773 ± 161pmol/L). proANP(1- 98) showed a significant correlation with NT-proBNP just before training session ($r=0.72$, $p<0.02$) in group A and just at the end in group B ($r=0.7$, $p<0.03$). During the training there were no cardiovascular problems. The ejection fraction and the left ventricular diameter remained unchanged in both groups (training and control groups) after 12 weeks. The training group showed was an improvement in general well being, in all-day activities and in fatigue as demonstrated by questionnaire (MLHFQ) whereas no improvement in the control group was observed.

Discussion and Conclusion: Present results suggest the importance of Natriuretic Peptide assays in confirming left ventricular maintenance and CHF follow-up during training session. Furthermore the different correlation of N-terminal fragment of ANP and BNP in group A before and after the session confirmed the

positive altered cardiac pattern secretion-exercise induced. This preliminary data could suggest: firstly, that this type of strength training is worthwhile in all-day activities; secondly, that BNP and NT-proBNP could be employed as maker of cardiac function worsening in failing of CHF rehabilitation, in order to "mark" when to start thinking of heart transplantation during CHF story.

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ADENOSINE METABOLISM AND APOPTOSIS IN EXPERIMENTAL LIVER TRANSPLANT

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Introduction: Organ dysfunction secondary to ischemia/reperfusion injury still represents a major problem in liver transplant. Besides necrosis, a potentially critical pathophysiological mechanism of the ischemia/reperfusion injury, is the development of apoptosis following liver transplant. Apoptosis has been observed in hepatocytes and sinusoidal endothelial cell (SEC) following ischemia-reperfusion injury and it has been postulated as a contributing factor in graft dysfunction following liver transplant. Moreover changes of protein tyrosine-kinase phosphorylation are involved in the regulation of apoptosis in various cell types [1]. The distinction between apoptosis and necrosis is blurred in the case of a pathologic insult such as ischemia/reperfusion. It could be of clinical benefit to learn how to differentiate them and fill the gaps in our understanding.
Materials and Methods: We studied 7 cases of experimental liver transplant; biopsies were obtained before explantation (t1), after cold ischemia (t2) and 30' from reperfusion (t3) Deproteinized samples were analysed by CE for purine compounds and glutathione [2]. The same CE method was utilised for the evaluation of some purine catabolic enzymes activities. We studied the behaviour of purine nucleoside phosphorylase (PNP), adenosine deaminase (ADA), and the two citosolic nucleotidases: AMP-ase (e-Ns) and IMP-ase (c-N-II). We evaluated the product formation in suitable incubation mixtures [3]. The Caspase-3 activity, DNA fragmentation and protein tyrosine phosphatase (PTP) activity were evaluated by specific commercial kits.
Results: High energy phosphates (HEP) concentrations and GSH/GSSG ratio significantly decreased after ischemia, (t2) adenosine and inosine monophosphate increased; after reperfusion HEP and IMP levels inverted the trend to decrease. ATP/ADP ratio was greatly reduced during ischemic phase and remained stable at reperfusion. Nucleotide/(nucleoside+base) ratio decreased at t2 but at t3 this tendency was reversed. GSH/GSSG ratio dramatically decreased in the time-course of transplantation. ADA and e-Ns were grossly reduced both at t2 and t3, PNP was increased at t3; no variations were evident for cN-II. C-3 activity showed a progressive increase from t1 (18.87 ± 7.11 nmol/h/mg prot.) to t2 and t3 (63.12 ± 10.15). In three cases, an increase in caspase-3 activity was associated with a sustained DNA fragmentation. PTP activity was increased at t2 and decreased at t3.
Conclusion: Data on HEP evidenced their active degradation to adenosine and IMP during ischemic time, a further degradation to inosine is however slowed down (confirmed from the ADA activity). This behaviour could be related to the preservation of AMP and adenosine, precursors of HEP via AK. PNP behaviour could be related to purine ring salvage through HPRT. We evidenced a deep imbalance of antioxidant status. The biochemical events associated with apoptosis include C-3 activation, DNA fragmentation and PTP reduction during the reperfusion. Dynamic alteration in PTK/PTP balance could be related to cell death through p38 protein (mitogen activated protein kinase MAPK) triggering [1]. Our results suggest that, during liver transplant, the complex series of insults results in a cell injury which can lead to apoptotic features. These findings could be of interest in new potential strategy to prevent and treat I/R injury.

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THE VIRTUAL SEGMENTAL BIOELECTRICAL IMPEDANCE ANALYSIS: A RELATIONSHIP BETWEEN AN ASSESSMENT OF LEFT VENTRICULAR END-DIASTOLIC VOLUME AND BODY EXTRACELLULAR WATER IN CHRONIC HEART FAILURE

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Introduction: Chronic Heart Failure (CHF) and homeostatic responses to haemodinamic injury have been the focus of numerous experimental clinical studies.
Objectives: To investigate the potential of "virtual" segmental Bioelectrical Impedance Analysis (BIA) for estimating changes of body water distribution in CHF patients. The BIA is a simple, reproducible method used to determine Total Body Water (TBW). Strengths of relationships were determined between indices of impedance or specific resistivies of body segments and reference heart function assessments of left ventricular Volume end-Diastolic (VTD) and Fractional Ejection(FE).
Material and Methods: 35 patients with chronic water imbalanced (e.g. peripheral oedema) and with CHF (mean age 47 ± SD22 years, left ventricular FE 30 ± 8%, New York Heart Association (NYHA) score 2.9 ± 0.3) were studied. Whole-body and segmental BIA were used to calculated impedance indices of arm, trunk and legs; TBW, Resistance (R) and Reactance (Xc) were assessed using a single-frequency (50kHz) tetra polar plethysmograph device according to Organ's procedure (1994). Echocardiography transtoracic standard parameters were evaluated.
Results: Mean TBW peaked in 1 day and declined thereafter on diuretic treatments. The patients were divided into two group: Group A with VTD > 150 and group B with VTD < 150 ml/mq. Mean arm, leg and trunk R and Xc were measured in 1 day and only trunk R was significantly lower in A than B group (21.25 ± 4.26 vs 25.46 ± 5.06 Ohm; $p < 0.04$, respectively). There is a positive linear regression between the extracellular water, in percentage of TBW, and left ventricular VTD in all patients ($r = 0.47$; $p < 0.05$). There was no significant correlation between trunk, arm and leg R and the absolute FE.
Discussion: The process of haemodinamic injury is not sufficiently specific for diagnosing patients with heart failure, however many general practitioners in European countries treat people with suspected heart failure on the basis of symptoms and signs alone. This study have identified in CHF patients with VTD > 150 ml/mq a greater truncal water distribution compared to patients with VTD < 150 ml/mq and this phenomenon is consistent with abnormal water changes in the body.
Conclusions: a) the present results suggest that virtual segmental bioelectrical impedance methods may be useful for predicting the body water distribution in CHF patients; b) the relationship between the extracellular water, in percentage of TBW, and left ventricular VTD might be a "risk marker" in management of CHF.

EXPRESSION OF ADHERENS AND TIGHT JUNCTIONS MOLECULES IN LIVER CIRRHOSIS: AN IMMUNOHISTOCHEMICAL ANALYSIS

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Background and aim: Liver cirrhosis is characterized by architectural rearrangement and changes involving molecules responsible for the cell-cell interaction. The aim of this study was to evaluate the expression and cellular localisation of the E-cadherin/catenin complex and the tight junction protein occludin in liver cirrhosis.
Materials and methods: An immunohistochemical study was performed on 14 liver biopsies obtained from patients with end stage liver cirrhosis (4 HCV, 4 HBV, 3 alcoholic, 1 primary biliary cirrhosis, 1 primary sclerosing cholangitis, 1 Wilson disease). Tissue sample obtained during surgery from a subject with normal liver function was used as a control. Immunostaining was evaluated and scored by extent and intensity.
Results: In patients with liver cirrhosis the expression and cellular localisation of E-cadherin was preserved in hepatocytes and the enhanced intensity of staining in the biliary duct epithelia was also maintained. Interestingly, increased E-cadherin expression was seen in regenerating hepatocytes. Compared to control, β-catenin expression was significantly increased, particularly in the subgroup with HCV related cirrhosis. However, no nuclear localisation was found. In the patient with primary biliary cirrhosis hepatocytes showed a reduced occludin expression.
Conclusions: The increased expression of E-cadherin seen in cluster of hepatocytes of highly regenerative areas could be consistent with their undergoing pseudoglandular and ductular transformation. The increased expression of β-catenin in HCV patients could be explained by the proproliferative gene profile induced by this infection. Further study in patients with early stages of primary biliary cirrhosis are required to confirm the possible role of occludin downregulation in the pathogenesis of this disease.

EFFECT OF NORMOTHERMIC ("WARM") HYPOXIA ON OXIDATIVE STRESS AND METABOLISM IN ISOLATED/PERFUSED RAT LIVER: HISTOCHEMICAL AND BIOCHEMICAL EVALUATIONS

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It is generally believed that the damage induced by "warm" ischemia is negligible with respect to that caused by reperfusion and that Reactive Oxygen Species (ROS) are the main effectors of tissue damage (Le Masters & Thurman, 1993). In order to check the presumed poor response and ROS production of the various liver cell populations to lack of oxygen and nutrients, we used histochemical and biochemical techniques to document the response of isolated rat liver perfused with N_2- or O_2-saturated medium at 37°C for up to 2 hours. We analyzed the perfusate for LDH activity released from dead cells and for thiobarbituric acid reactive species (TBARS) derived from oxidative stress and measured bile flow as index of liver metabolism. Assays were made every 10 min. Two significant time points of tissue damage (40 and 70 min) were selected to perform the histochemical assays. These comprised the demonstration of: (a) ROS production, with Kerver *et al.* (1997) Mn^{2+}-Co^{3+}-DAB technique; (b) the putative diaphorase activity of Nitric Oxide Synthase (NOS), with a technique improved in our laboratory (Freitas *et al.*, 2002); and (c) the activity of several enzyme activities linked to liver metabolism, namely Lactate Dehydrogenase (LDH), Succinate Dehydrogenase (SDH), Alkaline Phosphatase (AlkPh), Purine Nucleoside Phosphorylase (PNP) and Xanthine Oxidoreductase (XOR; comprising both the reductase and the oxidase forms of the enzyme), with standardized techniques (Van Noorden & Frederiks). Trypan Blue uptake was used as indicator of cell death. A scoring method was used to compare semi-quantitatively the response of hepatocytes, sinusoidal and biliary cells. Normoxic perfusion had negligible effects. By contrast, warm hypoxia caused lack of bile flow, hepatocyte staining by Trypan Blue in the midzone, alteration of the activity of all enzymes, increased ROS production by hepatocytes and sinusoidal cells (in keeping with high TBARS concentration in the perfusate) and release of LDH to the perfusate (in keeping with LDH loss from mid-zonal hepatocytes). ROS appeared to be produced mostly as by-products of the mitochondrial electron transfer chain in hepatocytes and during (auto)phagocytosis in sinusoidal cells and pericentral hepatocytes. NOS activity in Ito cells appeared to be correlated to sinusoid dilatation and PNP activity to be a sensitive marker of damage to sinusoidal and bile duct cells.
In conclusion, our results suggest that the vulnerability of the liver to warm hypoxia is lobular zone and cell type-dependent and that damage to the parenchyma is more serious than that expected from biochemical data alone. (Funds from FAR-UniPV; MIUR-COFIN 1999, 2001)

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HYPERHOMOCYSTEINEMIA AND IMMUNE ACTIVATION IN CORONARY HEART DISEASE

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Moderate hyperhomocysteinemia is considered to be a risk factor for coronary heart disease (CHD) and other vascular diseases. Folate and vitamin B12 are essential cofactors for the remethylation of homocysteine. According to this, usually an inverse relationship exists between serum concentrations of homocysteine and folate. Likewise, folate supplementation is able to lower homocysteine concentrations, so that hyperhomocysteinemia seems to develop from insufficient dietary intake of B vitamins. Inflammation and immune activation appear to be important in the pathogenesis of CHD, reflected by increased immune activation markers like C-reactive protein and neopterin. Neopterin is not only a marker of immune system activation, but additionally allows to estimate oxidative stress, which arises from reactive oxygen specie production of macrophages and other immunocompetent cells upon stimulation. Aim of this study was to investigate a possible relationship between immune activation and B vitamin status in CHD patients. We measured blood concentrations of homocysteine, B vitamins and neopterin in 35 patients with CHD verified by coronary angiography. Patients were observed before percutaneus transluminal coronary angioplasty (21 patients with one-artery disease, 9 with two- or three-artery disease, 5 with restenosis).
A significant proportion of patients presented with increased homocysteine concentrations, which coincided with lower folate (r_s= -0.469, $p< 0.01$) and also with higher neopterin levels (r_s= 0.472, $p< 0.01$). Also lower folate concentrations coincided with higher neopterin concentrations (r_s= -0.370, $p= 0.01$).
Data show that the increase of serum homocysteine is not only associated with lower circulating folate but also with higher neopterin concentrations, the latter being related to immune activation. Immune activation goes along with excessive production of reactive oxygen species and the development of oxidative stress. Methyl-tetrahydrofolate, the biologically active form of folate and an essential cofactor for the conversion of homocysteine to methionine, is very susceptible to oxidation. The inverse correlationbetween folate and neopterin concentrations would be in line with the assumption that oxidative stress could cause the degradation of tetrahydrofolate. Oxidative depletion of folate would then result in hyperhomocysteinemia, even when dietary folate intake is within the recommended range. Thus folate degradation and moderate hyperhomocysteinemia observed in patients with CHD could be an epiphenomenon of sustained immune system activation.

ANTI-HCV POSITIVE HAEMODIALYSED PATIENTS WAITING FOR KIDNEY TRANSPLANTATION: PROTECTIVE ROLE OF IL-10

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In anti-HCV positive haemodialysed patients (HD) ALT level is often normal and liver damage is mild. However it is crucial to evaluate the risk of liver disease progression due to immunosuppression after kidney transplantation. In this setting IL-10, a Th2 cytokine that modules the inflammatory responses, can play a role in regulation of hepatic fibrosis.

AIM: To assess IL-10 serum levels and correlate those with liver function tests (LFTs) and histological damage in anti-HCV positive HD patients waiting for kidney transpantation.

Methods: 71 anti-HCV positive HD patients, 58 anti-HCV negative HD patients, 41 patients with HCV chronic hepatitis and 20 healthy volunteers were enrolled in the study. In all patients LFTs and, when indicated, liver biopsy were performed. IL-10 serum levels were assessed by ELISA. Data are expressed as mean ± SE. Groups are compared by Student's T test.

Results: IL-10 serum levels were higher in anti-HCV positive HD patients (3.6± 0.42 pg/ml) and in anti-HCV negative HD patients (3.6± 0.82 pg/ml) compared to healthy volunteers (1.7± 0.4 pg/ml; $p<0.05$ and $p<0.01$ respectively). In anti-HCV positive HD patients the mean level of IL-10 was higher in those with normal ALT (3.9± 0.47 pg/ml) compared to patients with increased ALT (1.9 ± 0.48 pg/ml; $p<0.05$). In patients with chronic HCV infection IL-10 was 1.96 ± 0.36 pg/ml.

Liver biopsy was performed in 17 anti-HCV positive HD patients and in 18 patients with HCV chronic hepatitis. IL-10 serum levels in anti-HCV HD patients were higher in patients with mild histological damage compared to patients with moderate damage ($p<0.05$) whereas no correlation was found in HCV chronic hepatitis among different groups of severity of liver damage.

Conclusion: The haemodialysis treatment seems to stimulate the cytokine production in anti-HCV positive patients and the increased concentration of IL-10 protects the hepatocyte from necrosis and progression of liver damage. This may favour the outcome of liver function after kidney transplantation.

MONITORING OF CYSTATIN C LEVEL IN HEART TRANSPLANTATIONS - PILOT STUDY

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Cystatin C is a cysteine proteinase inhibitor with molecular weight 13,250 and is formed by all nucleated cells. As it is formed at a constant rate and freely filtered by healthy kidney it can be used for assessing renal function. Concentrations of cystatin C are almost totally dependent on the glomerular filtration rate (GFR). A reduction in the GFR causes a rise in the concentration of cystatin C. Cystatin C has not been shown to be affected by factors such as muscle mass and nutrition, factors which have been demonstrated to affect creatinine values. In addition, a rise in creatinine does not become evident until the GFR has fallen by approximately 50 %.

The aim of this study was to analyse values of cystatin C in comparison with creatinine levels in a group of heart transplantated patients (HTx).

Methods: We investigated plasma cystatin C and creatinine values obtained from a group of 24 patients (only males, with the mean age 46,71± 9,72) before and after heart transplantation (intervals 1,3,5,7,14,21 and 28 days after HTx). This study was performed in the Slovak Institute of Cardiovascular Diseases and St. Cyril and Methods Hospital, Bratislava, during the period of September 1999 - June 2003.

For determination of plasmatic levels of cystatin C we used diagnostic test kit N Latex Cystatin C (normal concentration 0.53-0.95 mg/l) from Dade Behring using BN II analyzer, creatinine was analysed using the casette Cobas Integra Creatinine Jaffe on analyzer Cobas Integra 700 (normal concentration male 80-133 umol/l). Both parameters were calculated to standard heamatocrite 0.40.

Conclusion: Our data show that in patients with heart transplants, cystatin C has similar diagnostic value as creatinine. Both parameters markedly increased compared to their preoperative values during the 3th and 5th day after transplatation (cystatin C 339%, creatinine 229%). 28 days after HTx the values were still increased (cystatin 190%, creatinine 134%). We can suggested that cystatin C is a more sensitive marker of kidney injury during heart transplantation.

COMPARISON BETWEEN A "MORE POINT CALIBRATION CEDIA" ASSAY, A 2 POINT CALIBRATION CEDIA ASSAY, AXSYM ASSAY AND LCMS-MS FOR CYCLOSPORIN A MEASUREMENT

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Background: Cyclosporin A (CY A) is widely used as a potent immunosuppressive drug. The monitoring of CY A is important because of toxic side effects, especially kidney function disorders. Because of the fact that the C0 levels show poor correlation with the exposure of CY A, the 2 hour post dose sampling (C2 monitoring) has been established. Therefore, an extended assay range for CY A is required. For this purpose Microgenics developed an assay (CEDIA PLUS) with two different calibration curves using aqueous standards with a low assay range from 25 - 450 µg/L and a high assay range from 450 - 2000 µg/L. Furthermore no separation step is needed. Since the original CEDIA PLUS is based on a 2-point calibration without a master curve, we established it on a HITACHI 911 using a 5 - and 4-point calibration, respectively.
Methods: A 5 point calibration for the low samples from 25 - 450 µg/L and a 4 point calibration for the high samples from 450 - 2000 µg/L were established using whole blood calibrators. 258 samples of kidney, liver, lung and stem cell transplanted patients were analysed with the "more Point calibration CEDIA" and compared with the AxSYM (ABBOTT). In addition 48 samples over 450 µg/L were investigated with the "4 point calibration CEDIA" and compared with the CEDIA-PLUS, AxSYM and LCMS-MS*
Results: The "Low Test Range Measurement" (25 - 450 µg/L) showed a good correlation (r= 0,9649 p<0,0001) and the mean values did not differ. (left Table). The "High Test Range Measurement" (450 - 2000 µg/L) showed a bit poorer correlation (r=0,86836 p < 0.0001) whereby the values were 14% higher at the CEDIA 4 point calibration compared to the AxSYM. (right Table)

n=191	MEAN (µg/L)	SD (µg/L)
AxSYM	187	97
CEDIA 5 point calibration	187	106
% of Difference (100%= Axsym Value)	99,1	19,5

n=67	MEAN (µg/L)	SD (µg/L)
AxSYM	832	285
CEDIA 4 point calibration	936	303
% of Difference (100%= Axsym Value)	114,1	20,0

As a result of this fact 48, samples higher than 450 µg/L were compared to the LCMS-MS.

n=48	MEAN (µg/L)	SD (µg/L)
AxSYM	766	315
CEDIA-PLUS	912	303
CEDIA 4 Point	769	277
LCMS-MS	669	269
AxSYM % of Difference (100%= LCMS-MS Value)	116,4	21,8
CEDIA-2 Point % of Difference (100%= LCMS-MS Value)	140,9	23,8
CEDIA-4 Point % of Difference (100%= LCMS-MS Value)	118,0	16,9

Correlation between LCMS-MS and Axsym: r=0,9376, p < 0.0001; Correlation between LCMS-MS and CEDIA-PLUS: r=0,94622, p < 0.0001; Correlation between LCMS-MS and CEDIA-4 Point: r=0,95825, p < 0.0001
Conclusion: Although, all methods showed a good correlation to the LCMS-MS, the results of the original CEDIA PLUS (2 point calibration) were 40% higher than the reference method, and 20% higher than the AxSYM and the 4 point calibration CEDIA.

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