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# Clinical Chemistry

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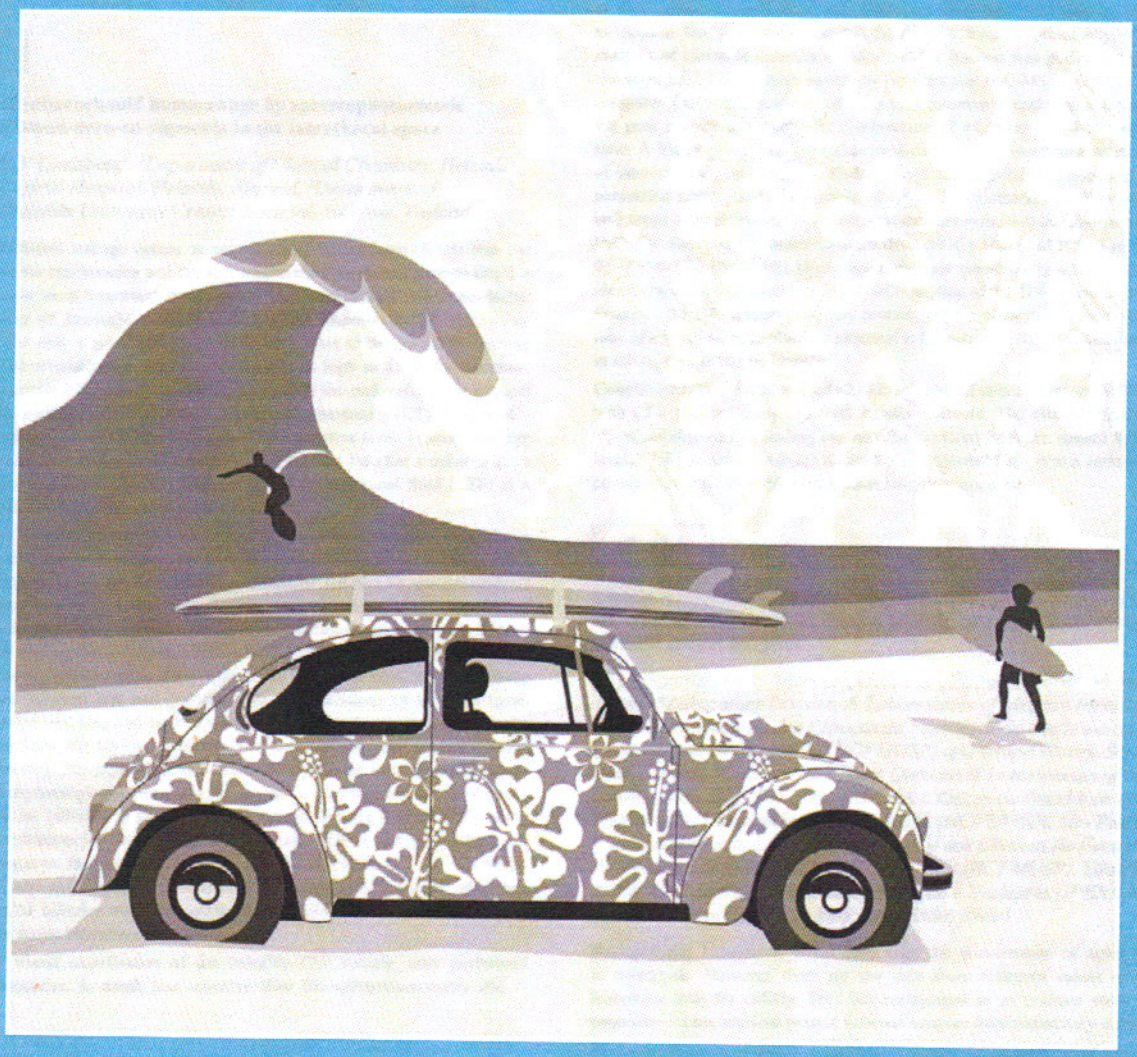

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performed on day 7. The mean time from the onset of stroke symptoms and hospital admission was 3.2 hours. Stroke severity was measured at the time of admission with the Scandanavian Stroke Scale (SSS). Functional outcome was measured with the modified Rankin scale (mRS) on day 7 and acute stroke patients were categorised into three severity groups (mild, moderate and severe) according to their mRS-score: mild (mRS-score:0-2), moderate (mRS-score:3-4) and severe (mRS-score:5-6). GFAP levels were quantified in EDTA plasma samples employing Randox-BAT technology on the Evidence Investigator analyser.

**Results:** The mean age (SD) of the patients was 75.2 (9.4) years. Forty-two patients (42.86%) died during a follow-up period of 1 year. The mean time (SD) between the onset of neurological symptoms and hospital admission was 3.22 (1.58) hours. At admission, mean plasma GFAP levels were significantly elevated in ICH (10.40ng/ml) compared to IS patients (0.17ng/ml) and healthy controls (0.12ng/ml) (P<0.0001 anova-test). The diagnostic accuracy of a single GFAP measurement upon hospital admission for the differentiation between patients with ICH and those with IS, is high [AUC=0.87 (95%CI 0.72-0.95), P<0.0001]. Further analysis revealed that GFAP values within the ICH group were significantly higher among patients with a higher severity score (14.91ng/ml) compared to moderate (1.29ng/ml) and mild (0.37ng/ml) groups (p<0.0001 anova-test). No such differences were observed within the IS group 0.21 vs 0.17 vs 0.14ng/ml respectively). Furthermore, within the ICH group GFAP levels were significantly lower among survivors (1.27ng/ml) when compared to non-survivors (14.70ng/ml) (p<0.005) while no such differences were observed within the IS group of patients (0.17 and 0.16 respectively).

**Conclusions:** Our data suggest that the determination of GFAP levels upon admission can be used to differentiate between IS and ICH as well as serve as a predictor of severity and mortality among ICH patients.

## E-184

### Diagnosis of subarachnoid hemorrhage by spectrophotometric detection of blood-derived pigments in the intrathecal space

L. Uotila[1], P. J. Lindsberg[2]. *[1]Department of Clinical Chemistry, Helsinki University Central Hospital, Helsinki, Finland, [2]Department of Neurology, Helsinki University Central Hospital, Helsinki, Finland*

**Background:** Blood leakage occurs in subarachnoid hemorrhage (SAH) into the space between the arachnoidea and the soft membrane (pia mater) surrounding the brain. The single most important symptom of SAH is a sudden severe headache. A large number of headache patients seek medical attention in the emergency departments, but only a small part turn out to have SAH or the so-called warning leak into the intrathecal space. Mortality in SAH is as high as 40-50 %. Accurate, immediate diagnosis is therefore imperative to expedite the endovascular or surgical treatment of the possible arterial aneurysm. Computed tomography (CT) of the head is the principal diagnostic method but may give a false negative result in small warning leaks or if the patient is coming to the emergency department after a delay of days or even weeks. Spectrophotometric analysis of the cerebrospinal fluid (CSF) is a sensitive diagnostic technique under these conditions.

**Methods and Results:** We report a series of 772 spectrophotometric CSF analyses performed in possible SAH cases in the Helsinki University Central Hospital during 2007-2011. Physicians had been instructed to submit CSF samples for spectral analysis when the patient was suspected to have SAH or a warning leak despite negative or unclear CT findings, or when it was deemed necessary to rule out an underlying SAH in unequivocal or nonspecific symptomatic clinical diagnoses such as sudden headaches. We used for the analyses an autoscaling Agilent 8453 spectrophotometer by scanning the absorbance between 600 and 350 nm. The concentrations of bilirubin (peak absorbance at 450-460 nm) and oxyhemoglobin (peak absorbance at 415 nm) were determined. In SAH, the CSF concentrations of both bilirubin and oxyhemoglobin are usually elevated. Elevated bilirubin is clinically significant even alone, but small elevations of oxyhemoglobin alone usually suggest a puncture artifact. The upper reference limit for bilirubin in the CSF is 0.17 micromol/l; we could reliably assay bilirubin concentrations well below that. In the majority of the cases (90 %) the result was clearly negative. In thirtythree cases (4.3 %) the diagnosis of SAH was made. Possible SAH was encountered in fifteen additional cases (1.9 %). In 20 additional cases (2.6 %) CSF bilirubin was increased but was explained by the increased level of CSF protein or serum bilirubin or both.

**Conclusions:** Visual examination of the valuable CSF sample, only performed by many laboratories, is much less sensitive than the spectrophotometry and is discouraged.

## E-185

### Search for a novel salivary biomarker candidate for with chronic fatigue syndrome

J. Kalns[1], D. Michael[1], T. Whistler[2], B. Valle[1]. *[1]Hyperion Biotechnology, Inc., San Antonio, TX, [2]Hyperion Biotechnology, Inc., Centers for Disease Control, GA*

**Objective:** Identification of a salivary biomarker candidate that could be explored further for its ability to diagnose chronic fatigue syndrome (CFS). Relevance to clinical laboratory medicine: At present, there is no objective method to diagnose CFS. The current method of diagnosis requires the physician to rule out all other possible causes of chronic fatigue before assigning a diagnosis of CFS. An objective and rapid method to diagnose CFS would reduce the time required to arrive at a definitive diagnosis.

**Methods:** Saliva was obtained from an archive of samples originally collected as part of a study conducted in the State of Georgia, USA by the Centers for Disease Control and Prevention (Reeves, et al. BMC Health Services Research, 2009, 9:13) to determine prevalence of CFS in the community. Briefly, 10,837 households were contacted through telephone survey. A total of 780 individuals participated in clinical assessment. Based on clinical assessment, 112 participants were found to have CFS. Healthy controls were composed of 147 subjects from the study that did not report signs of fatigue on initial interview and later were found to be free of symptoms of CFS or other significant medical conditions. As part of the clinical assessment saliva samples were obtained for biochemical assessment. Saliva samples were maintained at -80C until evaluated. Raw saliva samples were first processed by spin-filtering to acquire the small-molecular-weight (sMW) fraction (nominally, <5kDa). An analysis of ionizable components in the sMW fraction was performed using liquid chromatography with mass spectrometric detection (LC-MS). LC-MS results were compared first within groups to identify ions common to each group, i.e. a significant ion peak present at a particular combination of retention time and mass-to-charge ratio. A list of promising biomarker candidates was reduced to a single biomarker of interest based upon the significance level of the difference observed for a non-parametric comparison of the group samples. This biomarker candiate was found to be 3 times more abundant in CFS subjects than controls (p<0.001, Wilcoxon rank sum test). The Receiver Operating Characteristic (ROC) Area-Under-the-Curve (AUC) is 0.94 (95% CI 0.86 to 0.98). High-resolution mass spectrometry was used for chemical identification of this candidate as a 2.6kDa peptide of the 42-kDa basic Proline-Rich-Protein 4 (PRB4), a known salivary protein. MS-fragmentation pattern and retention time of a synthesized peptide are identical to the native biomarker candidate identified in saliva, confirming its identity.

**Conclusions:** We have identified a 2.6kDa peptide of salivary protein PRB4 associated with CFS patients compared with healthy controls. The clinical significance and reproducibility of this finding can now be explored in future studies by comparing levels of the specific candidate biomarker in a blinded fashion in a variety of patients populations, including those with other fatiguing illnesses.

## E-186

### Characterization of biochemical parameters, trace elements and hormones concentrations from a healthy elderly population of a medical school hospital.

N. M. Sumita[1], M. E. Mendes[2], O. Jaluul[3], W. Jacob Filho[3], M. Saiki[4]. *[1]Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP) and Grupo Fleury, São Paulo, Brazil, [2]Central Laboratory Division & Laboratories of Medical Investigation (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, [3]Clinical Geriatric Discipline of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, [4]Instituto de Pesquisas Energéticas e Nucleares (IPEN), Neutron Activation Analysis Laboratory, São Paulo, Brazil*

**Background:** Nowadays, it has been seen the phenomenon of aging population in worldwide. However, there are few data about reference values to be used in laboratory tests for elderly. This fact encouraged us to evaluate some laboratory parameters concentrations present in blood samples from ambulatory elderly people.

**Methods:** A group of elderly population without clinical evidence of serious chronic diseases was evaluated. The study was submitted and approved by our Internal Review Board (IRB). The patients were selected based on the SENIEUR protocol

(*SENIor EURopean Protocol*). The blood samples of 120 elderly people (34 men and 86 women), aging 72 ± 8 years, were analyzed. The blood, after 12 hours fast, was collected by venipuncture using sterile standard metallic needles. It was collected in two types of evacuated tubes (Vacutainer Systems - Becton Dickinson, EUA): SST II *Advance* gel and clot activator tube and a specific tube for trace elements analysis, without heparin. Biochemical analyses were carried out on Roche/Hitachi MODULAR *ANALYTICS* PP (Roche Diagnostics GmbH, Germany). The neutron activation analysis (NAA) was applied for trace elements determination. Short and long irradiations were carried out under a thermal neutron flux of about 4 x $10^{12}$ n $cm^{-2}$ $s^{-1}$ for Br, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using a Hyperpure Ge detector Model GX2020 coupled a gamma-ray spectrometer. The radioisotopes measured were identified according to their half-lives and gamma-ray energies and the element concentrations were calculated by comparative method. The thyroid hormones were evaluated on AutoDELFIA automatic immunoassay system (Perkin Elmer, USA). The HPLC method certified by NGSP was used to measure the glycated hemoglobin level on Variant II turbo (Bio-Rad Laboratories, Inc., USA).

**Results:** Biochemical parameters: uric acid: 5.1±1.4 mg/dL, total bilirrubin:0.71±0.26 mg/dL, Na:141±3 mEq/L, K:4.5±0.4 mEq/L, Ca:9.5±0.5 mg/dL, ionized Ca:5.1±0.5 mg/dL, P: 3.5±0.5 mg/dL, Mg:2.10±0.28 mg/dL, glucose:93±10 mg/dL, glycated hemoglobin:5.7±0.5%, urea:37±13 mg/dL, creatininc:0.84±0.19 mg/dL, Fe:105±31 µg/dL, total iron-binding capacity:301±38 µg/dL, ferritin:183±155 ηg/mL, total protein:7.3±0.5 g/dL, albumin:4.4±0.3 g/dL, total cholesterol:211±36 mg/dL, HDL-cholesterol:59±15 mg/dL, LDL-cholesterol:128±32 mg/dL, triglycerides:122±61 mg/dL, AST:22±7 U/L, ALT:20±11 U/L, alkaline phosphatase:80±25 U/L and GGT:23±13 U/L. Trace elements: Br: 3.46± 0.85 mg/L, Rb: 320.0± 56.9 µg/L, Sc: 77.0±25.3 µg/L and Zn 95.2±13.6 µg/L. Thyroid hormones: TSH:2.09±1.60 µU/mL, total thyroxine:8.9±1.7µg/dL, total triiodothyronine:122±19 ηg/dL and free thyroxine:1.08±0.21ηg/dL.

**Conclusions:** The results suggest the need to establish specific reference values for elderly. The population evaluated do not present deficiency or excess of trace elements.

## E-188

### Lower sex hormone-binding globulin (SHBG) is independently associated with metabolic syndrome in middle-aged and elderly men in China

X. N. Pang[1], Y. Yuan[1], J. P. Shen[1], Y. Hu[1], X. Sun[2]. [1]*Department of Geriatrics, Zhongshan Hospital, Fudan University, Shanghai, China.* [2]*Beckman Coulter Commercial Enterprise (China) Co. LTD., Shanghai, China*

**Objective:** A low level of testosterone in men has been shown to be related with an increased risk of metabolic syndrome. Free testosterone, the main active form of testosterone, is not always tested directly. Because sex hormone-binding globulin (SHBG) increases with age while testosterone declines, we examined the relationships between SHBG and the metabolic syndrome in middle-aged and elderly men in China.

**Methods:** A cross-sectional study was done among 437 men, aged 45 to 94, from the health checkup population of Zhongshan Hospital Fudan University. Patients who were under treatment with hormone replacement therapy (HRT), or diagnosed with thyroid disease, chronic renal failure, chronic hepatopathy or cancer, were excluded from the study. Early morning fasting sera were assayed for total testosterone, SHBG, and other biochemical markers such as fasting glucose, fasting insulin, TC, HDL-C, LDL-C, TG. SHBG was measured using a chemiluminescent immunoassay (Beckman Coulter). Free testosterone was calculated using the Vermeulen equation (Vermeulen, JCEM 1999). Complete medical history was taken and reviewed for each patient.

**Results:** Metabolic syndrome was defined using the criteria of the Chinese Diabetes Society (CDS 2004). There were 82 men with metabolic syndrome (18.7%). The SHBG level of the metabolic syndrome group was significantly lower (39.27±26.54nmol/L, p=0.030) than that of non-metabolic syndrome group (45.49±20.32nmol/L). SHBG correlated significantly with systolic BP (Spearman r=-0.020), diastolic BP(r=-0.100), BMI(r =-0.350), and FBG(r=-0.096), with all p-values < 0.05. Analyzing the effect of SHBG on parameters of the metabolic syndrome in a linear multivariate regression analysis, adjusted for age and insulin resistance index (HOMA-IR = Fasting Glucose (µU/ml) ×Fasting Insulin (mmol/L)/22.5), SHBG was significantly and inversely associated with systolic BP, diastolic BP, BMI and FBG, while HDL-cholesterol and triglycerides showed no significant relationship to SHBG after adjusting for HOMA-IR and age. In a logistic regression taking metabolic syndrome as the dependent variable, age, SHBG and HOMA-IR were included in the final model with statistical significance.

**Conclusions:** Lower SHBG is independently associated with metabolic syndrome among middle-aged and older men. SHBG may be an independent predictor of metabolic syndrome, but the mechanism of how SHBG is involved in the metabolic syndrome needs to be further studied.

## E-189

### Nuclear factor-kappa B activity in the peripheral blood lymphocytes measured with the novel quantitative system using the fluorescent correlation spectroscopy correlates with intra-abdominal fat area in patients with essential hypertension

K. Harada[1], S. Mikuni[2], F. Tomoda[1], S. Kagitani[1], T. Koike[1], M. Kinjo[2], H. Inoue[1], I. Kitajima[1]. [1]*University of Toyama, Toyama, Japan,* [2]*Hokkaido University, Hokkaido, Japan*

**Background:** Etiology of essential hypertension is extremely complex, and obesity and insulin resistance are its important risk factors. They are now widely recognized as chronic inflammatory diseases. The transcription factor, nuclear factor-kappa B (NF-κB) is known as a key regulator of many inflammatory processes. NF-κB regulates the expression of various inflammatory cytokines involved in metabolic diseases. We hypothesized that NF-κB activity could be the useful indicator of systemic inflammation in patients with hypertension. However, convenient assay systems to measure the NF-κB activity quantitatively in a timely manner have not been available in the setting of a hospital laboratory. Therefore, we have established a novel measurement system for the NF-κB activity using fluorescence correlation spectroscopy (FCS). We used this method to evaluate NF-κB activity and examined the correlation of NF-κB activity with other clinical parameters in 45 untreated patients with essential hypertension.

**Methods:** FCS was a methodology to examine the size and number of fluorescent-labeled molecules in a confocal area on the basis of the fluctuation of fluorescent intensity by their Brownian motion in solutions. The principle of NF-κB quantitation was to analyze the difference of the fluctuation of fluorescent intensity between the fluorescent-labeled DNA probe and fluorescent-labeled DNA probe NF-κB complex. We collected peripheral blood lymphocytes from 45 patients (mean age±SD: 51.8±13.1) with hypertension, who have no sign of renal dysfunction (eGFR > 60mL/min/1.73m2), and examined the NF-κB activity in the nuclear extracts of lymphocytes. We assessed the correlation between NF-κB activity and other biomarkers by multiple stepwise regression analysis and logistic regression analysis.

**Results:** NF-κB activity in patients with essential hypertension (mean±SD: 0.18±0.16 ng/µg of nuclear protein) was positively correlated with daytime systolic blood pressure, diastolic blood pressure, serum total cholesterol levels, triglyceride levels, serum lactate levels (The Pearson correlation coefficients = 0.319, 0.357, 0.310, 0.428, 0.438, and P values = 0.047, 0.026, 0.038, 0.003, 0.004, respectively). Multiple stepwise regression analysis revealed that intra-abdominal fat area was independently correlated with NF-κB activity ($p< 0.05$, R2=0.66, AICc=-9.86). Eighteen out of 45 patients were diagnosed with metabolic syndrome. NF-κB activity in patients with or without metabolic syndrome was 0.26±0.19 and 0.12±0.12 ng/µg of nuclear protein, respectively. In logistic regression analysis, NF-κB activity was significantly correlated with patients with metabolic syndrome ($\chi2=9.42$, $p<0.05$).

**Conclusions:** These results suggest that the elevation of NF-κB activity reflects the systemic inflammation caused by active visceral fat in the patients with hypertension. The development of this assay system using the FCS technique enables us to rapidly measure the NF-κB activity with high sensitivity in nuclear extracts of lymphocytes. Measuring the NF-κB activity in the patients with essential hypertension would be useful as an inflammatory marker to evaluate patients with metabolic syndrome-associated hypertension.

## E-190

### Assessment of Cystatin C as an Index of Allograft Function in Kidney Transplantation

H. Ucar, H. S. Akbas, V. T. Yilmaz, A. Aktas, G. Suleymanlar, G. Yucel. *Akdeniz University, Faculty of Medicine, Antalya, Turkey*

**Background:** Management of renal transplant patients requires periodic measurement of renal function, which is usually assessed by measuring the glomerular filtration rate (GFR). Serum concentration of Cystatin C (CyC) has been proposed as a marker to determine a reduced GFR, but the data of its value in renal transplant patients are conflicted. Urine CyC can be considered a marker of proximal tubular function and has been described its utility for predicting allograft function. This prospective observational study aimed to assess the relevance of serial postoperative serum and urinary CyC measurements for predicting allograft function after kidney