P07 – Endocrinology

P07-01

Indirect estimation for reference intervals of thyroid parameters

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Background: Reference intervals are essential for clinical laboratory test interpretation and patient care. Methods for estimating them are expensive, difficult to perform and often inaccurate. To establish indirect reference intervals from intra-laboratory data bases is an adequate alternative.

Materials and methods: All results for thyrotropin (TSH), total and free thyroxine (T4 and fT4), total and free triiodothyronine (T3 and fT3) that were stored in our laboratory information system between 2008 and 2011 were included in this study. After a logarithmic transformation of the raw data, outliers were excluded. Non-parametric reference intervals were estimated statistically after visual observation of the distribution using stem-and-leaf plots and histograms. A standard normal deviation test was performed to test the significance of difference between sub-groups.

Results: We calculated combined reference intervals for all thyroid parameters because there was no significant difference in serum values between male and female. However, we found significant difference for TSH values between ambulatory and hospitalized patients only in 2011. Indirect reference values for TSH, T4, fT4, T3 and fT3 were 0.42-3.67 mIU/L, 66.0-136.10 nmol/L, 10.20-18.40 pmol/L, 1.10-2.39 nmol/L, 3.17-5.59 pmol/L, respectively.

Conclusions: Indirect determination for laboratory-specific reference intervals using patient laboratory data values is relatively easy and cheap method. Also indirect reference intervals are more precise and true reference values of thyroid parameters for the analyzed population.

P07-02

Does TSH within normal range affect bone turnover in postmenopausal women with osteoporotic fracture

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Background: Recent studies indicated the existence of the relationship between TSH and fractures independently of thyroid hormone levels, age and bone mineral density. We aimed to evaluate the association between serum TSH and biochemical bone turnover markers in postmenopausal women with normal thyroid function, which suffered osteoporotic fracture, to answer whether the differences in TSH concentration within the reference range may affect bone metabolism.

Materials and methods: 36 women (55-93 years old) admitted to the Hospital after osteoporotic hip fracture participated in the study. Serum propeptide of type I procollagen (PINP; for postmenopausal women < 45.0 ng/mL), a bone formation marker and crosslinked C-terminal telopeptides (CTX-I; cut-off 0.439 ng/mL), a bone resorption marker and TSH were assayed. Study group was divided in two groups according to age: 55-70 and 71-93 yrs.

Results: In the older group a tendency to higher TSH concentration (Me 0.98 *vs*. 0.56 mIU/mL; $P < 0.06$) and higher median PINP (35.6 *vs*. 26.7 ng/mL; $P < 0.004$) was observed. PINP correlated negatively with TSH ($r = -0.44$ $P < 0.04$) only in the older group. Interestingly, most of women with fractures ($N = 25$; 69.4%), independently of age, had TSH concentration in the first tertile (0.36-1.38 mIU/mL) whereas only 2 (5.6%) had TSH in the highest tertile (2.4-3.41 mIU/mL). In both age groups median PINP, but not CTX-I, was the highest in patients with TSH in the first tertile.

Conclusion: In postmenopausal women TSH concentration within the reference range seems to be associated with changes in bone turnover and frequency of fractures independent of age.

P07-03

Serum calcium: much more than "bones"

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Introduction: The giant parathyroid adenoma manifests in different forms: from asymptomatic to hypercalcaemic crisis, a rare endocrine disruption.

Case: The authors present the case of a 62 year-old man in bad general condition, asthenia, polyuria, and polydipsia with 2 months of evolution. Later the patient revealed intense pain in the lumbar spine, and presented at the emergency service. He was conscious, oriented and revealed slow speech, memory disorders and hypertension (186/98 mmHg). In analytical terms he showed: severe hypercalcemia (total serum calcium = 20.0 mg/dL), the highest value ever registered in our laboratory; ionized calcium of 2.81 mmol/L; normal albumin levels, renal insufficiency (creatinine = 3.9 mg/dL, urea = 105 mg/dL); hyperphosphataemia 5.2 mg/dL. Calcium levels decreased significantly with strong hydration and the administration of bisphosphonates. There was an adequate diuretic response, with gradual improvement of the renal function. The cervical ultrasound scan described multinodular goiter, voluminous nodule of 32 x 27 mm at the left inferior pole and PTH = 1000 pg/mL. The patient underwent an urgent cervical surgery with exeresis of the giant parathyroid adenoma (21 grm; 3cm its largest axis) and treatment to restore normal levels of serum calcium by post-surgery "hungry bone". The patient progressed, showing clinical and analytical improvement.

In this case, even though the huge serum calcium values suggested the possibility of neoplasic/metastatic disease, the final diagnosis was primary hyperparathyroidism due to the giant parathyroid adenoma that was promptly treated.

P07-04

Tear cytokines in Basedow-Graves ophthalmopathy

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Background: Human tear is rich in cytokines. Even in healthy individuals the cytokine:total protein ratio is higher than that in serum. However, in Basedow-Graves disease an elevation of serum cytokine levels was found and also an altered cytokine response of the intra- and periorbital tissues was postulated. In our present study we focused on the measurement of IL-6, IL-8 and TNF-α levels and the amount of a lipocalin-type protein (orosomucoid) in tear samples of Graves patients.

Matrials and methods: Tears were collected by the Schirmer method from 35 patients and 20 control individuals without autoimmune and thyroid diseases. 80% of them were females. The mean age was 45 (25-71) years. The total tear protein content was measured by the Bradford method and tear proteins were separated by SDS-PAG electrophoresis. Tear orosomucoid was detected by chemiluminescent Western blot technique. Tear cytokine levels were quantified by an automated chemiluminescence immunoassay method (Immulite, Beckman-Coulter).

Results: TNF-α and IL-6 increased significantly (mean ± SEM, $P < 0.01$) in tear samples of the patients (TNF-α: 513 ± 57 *vs*. 217 ± 27pg/mg, IL-6: 39 ± 6 *vs*. 12 ± 1.2 pg/mg protein, patients *vs*. control in-

dividuals, respectively). In contrast, IL-8 and orosomucoid levels were similar in the two groups.

Conclusions: In our opinion increased tear TNF-α and IL-6 levels are considered to be a new finding in Graves patients. Further studies are needed to decide if tear cytokines might be used in assessment of activity of this disease and/or to monitor the efficiency of treatment. The work was supported by SROP-4.2.1.B-10/2/KONV-2010-0002 grant.

P07-05

Determination of total prolactin and prolactin treated with 25% PEG

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Introduction: Prolactin is a hormone of the anterior pituitary gland and occurs in human serum in several molecular forms: monomer (predominant form), big-PRL (dimer) and big-big-PRL or macroprolactin (compound of PRL and IgG), which is biologically *in vivo* inactive and biologically active *in vitro*, and leads to apparent hyperprolactinemia leading to inadequate diagnosis, treatment and diagnostic procedures.

Objective: Our objective was to compare the values of prolactin by two different immunochemical methods and examine their sensitivity to macroprolactin by determination of prolactin in the supernatant after treating the sample with 25% PEG solution.

Materials and methods: The study included 33 patients (31 women and 2 men) with elevated prolactin values. Values of total prolactin and prolactin after precipitation with 25% PEG were determined by immunoassay on analyzers COBAS e601 (Roche, USA) and i2000SR ARCHITECT (Abbott, USA).

Results: High values of prolactin were obtained and confirmed by both immunochemical analyzers but the COBAS e601 indicates greater sensitivity to macroprolactin. If the criterion of acceptance for macroprolactin is PRLtreated / PRLtotal < 40% then macroprolactin in the study group was found in 3 patients (27%, 19% and 38%) which has been confirmed by both methods.

Conclusion: Based on the results of this study we conclude that the measured values of prolactin are above the upper limit of the reference interval on both analyzers and that COBAS e601 exhibits greater sensitivity to macroprolactin, which is consistent with literature data.

P07-06

Reference intervals for reproductive hormones in prepubertal children on the Cobas e 411 analyzer

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Background: Serum values of luteinizing hormone (LH), follicle-stimulating hormone (FSH), gonadal hormones, as well as sex-hormone binding globulin (SHBG) levels in children of prepubertal ages are essential biochemical markers used for the evaluation of suspicious disorders of sexual development. The aims of this study were to establish reference intervals for LH, FSH, E2, P, T (total and free) and SHBG in prepubertal children according to age and gender and to assess age- and gender-related differences. We used the most recent guidelines of Clinical and Laboratory Standards Institute (CLSI) to establish and verify the reference intervals.

Materials and methods: A total of 966 children, 495 girls and 471 boys, between 1 to 11 years have been participated in the study. The hormone concentrations were measured by electrochemiluminscence immunoassay on the Automated Roche cobas e 411 Analyzer. The 2.5th and 97.5th percentiles are used to form the 95% reference limits.

Results: Median values of LH, FSH and T were significantly higher in subgroups ranging from ≥ 8 to < 11 years, for both genders. In girls of that age, reference values of E2 were significantly higher than in younger ones, and in boys of the corresponding age.

Conclusions: We provided reliable reference intervals for hormones relevant for the assessment of pituitary-gonadal axes in healthy children of prepubertal age. The reference intervals have been derived out of population large enough to create age subgroups which are appropriate to express subtle changes of hormones from early childhood to the period preceding the onset of puberty.

P07-07

Seasonal variation of neonatal thyrotropin values

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Background: Seasonal variation in incidence of higher thyroid stimulating hormone (TSH) values in newborns has been suggested in number of geographical areas and not observed in others. Aim of this study was to evaluate seasonality of TSH levels in our National newborn screening program for congenital hypothyroidism.

Materials and methods: TSH levels were measured in all neonates between third and sixth days after birth using a blood spot assay (DELFIA Neonatal TSH assay, PerkinElmer/Wallac Oy). The data from three consecutive years were available, 2008-2010, when 24515 newborns were screened. Children with TSH values ≥ 10 mU/L were recalled for measurement of serum TSH and fT4.

Results: Recall rate for chosen time period was 1.13%, 0.97% for 2008, 1.36% for 2009 and 1.06% for 2010. Positive predictive values were 2.53% for 2008, 7.08% for 2009, 3.49% for 2010 and 4.68% for all three years. Frequency of newborns with TSH levels ≥ 10 mU/L was higher in all childbirth centers in winter comparing to summer periods. The incidence was significantly higher in january and february (1.02%) compared to july and august (0.62%), P < 0.05. Recall rates were lower in summers (0.92%, 0.34% and 0.55%) compared to winters (1.27%, 0.94% and 1.35%) for all three years.

Conclusion: Influence of environment factors on thyroid physiology was proven from many studies. There is no data regarding urinary iodine excretion over different seasons for our population but it is possible that different iodine intake during periods can contribute to seasonality of neonatal TSH values.

P07-08

Relationship between body mass index, mercury and selenium levels in a hospital working population

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Background: Body mass index (BMI) is a simple weight-for-height index that is commonly used to classify underweight, overweight and obesity in adults. The objective of this study is to explore the relationship between blood lead and mercury and serum selenium concentrations and the BMI.

Materials and methods: We recruited 395 employees (64 men and 331 women). Blood mercury concentration (µg/L) was measured by atomic absorption spectrometry and thermal decomposition amalgamation. Serum selenium concentration (µg/L) was measured by electrothermal atomic absorption spectrometry. BMI is defined as the weight in kilograms divided by the square of the height in metres (kg/m^2).

Results: The median of blood mercury was 8.00 µg/L (IQR: 5.20-11.60). The mean of serum seleni-

um was 79.5 µg/L (SD: 11.7). The mean BMI of the population was 24.4 kg/m^2 (SD: 3.9). No significant differences were found between BMI and mercury ($r = 0.083$; $P = 0.116$) or between selenium and the selenium/mercury ratio. Considering BMI subgroups (< 25, 25-30 ≥ 30), we found a statistically significant ($P = 0.014$) increase in serum selenium concentrations (78.5 SD: 12.1; 79.8 SD: 10.7; 85.0 SD: 11.4 respectively). However, the increase observed in blood mercury concentration and selenium/mercury ratio was not significant.

Conclusions: In spite of the relation between mercury and selenium concentrations, with respect to BMI, we only found an association with serum selenium. Further studies are needed to understand the role that these trace elements, present in certain nutrients such as fish, may play in overweight and obesity.

P07-09

Is there any justification in the increasing demand for thyroid tests in our laboratory?

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Introduction: Thyroid disorders are among the most prevalent problems in clinical endocrinology. A majority of these patients are managed in primary care. The aim of our study was to evaluate the diagnostic performance of the increased thyroid test requests in the Health Area V of Asturias (Spain) during the last 9 years.

Materials and methods: Data were retrospectively collected by reviewing analysis of TSH, FT4 and FT3 during the last 9 years (2003-2011), from our Laboratory Information System Lmx (Siemens). These were divided into 2 groups: Group1: requests by Endocrinology Department and Group2: hospitalization, Emergency-Room and Primary Care.

Results: Progressive increase in thyroid test requests, from 44.932 TSH (2003) to 78.560 (2011), represents 84% of overall increase, which is not justified by the population increase experienced in our Area (2.75%). While the number of requests from the group 1 remained stable over the years (4.928 in 2003 and 4.811 in 2011), requests from the group 2 increased significantly (40.004 to73.749). Percentage of pathological TSH remains relatively stable at around 15% (13.8% to15.3%). Except in subclinical hypothyroidism, where its prevalence has grown from 4.94% to 10.8%, prevalences of other thyroid disorders remained constant: clinical hypothyroidism (1.28% to 0.82%), clinical hyperthyroidism (0.91 to 0.42%) and subclinical hyperthyroidism (3.89% to 2.71%).

Conclusions: We found that the increase of 84% of TSH is not then reflected in increased pathology in the endocrinology department because the pathology that increases is essentially subclinical hypothyroidism (Δ5.85%), whose patients are followed by primary care. Prevalence of thyroid diseases reported here is substantial and confirms previous reports in other populations.

P07-10

Diagnostic value of anti-thyroglobulin antibodies in the detection of thyroid pathology in children

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Background: The determination of anti-thyroid peroxidase antibodies(anti-TPO) is the most specific and sensitive test for detecting autoimmune thyroid disease. Anti-TPO are commonly associated with Hashimoto's thyroiditis (90%) and Graves disease (70%), an antibody titer can be used to assess disease activity in patients that have developed such antibodies. Thyroglobulin antibodies (anti-TG) are mainly used for monitoring thyroid cancer. The determination of anti-TG in adults is

only done in the suspicion of interference in thyroglobulin measurement and thyroid scintigraphy studies. The aim of our study was to establish the diagnostic value of the isolated use of anti-TG in the pediatric population of our country.

Materials and methods: We retrospectively studied 348 samples with anti-TPO and anti-TG analysis, made during the last 6 years in children with suspected thyroid dysfunction or thyroid autoimmune disease mother (mean 12 years, range: 1 day-13 years). Data were extracted from our computer laboratory manager Lmx (Siemens). Tests were performed by fluorinmunoanalisis (Phadia Unicap).

Results: Of all requests reviewed, 34 (9.77%) had positive anti-TPO (>60 U/mL): of these, 18 were anti-TG positive (> 280 U/mL) and 16 negative. In addition, 11 (3.16%) were anti-TPO negative and anti-TG positive. In reviewing the medical history of patients with anti-TPO negative and high anti-TG, we observed that 100% had thyroid disease: 18.18% autoimmune thyroid disease inherited by the mother, 63.64% euthyroid autoimmune thyroiditis and 18.18% subclinical hypothyroidism.

Conclusions: According to the results, the quantification of anti-TG rises by 32.3% the diagnostic yield of childhood thyroid diseases (34 positive anti-TPO positive+11 only positive anti-TG) which would justify the joint determination of both antibodies in the population study.

P07-11

Testosterone quantification in human serum using liquid chromatography with tandem mass spectrometry

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Background: Evaluation of testosterone levels is important for the diagnostic and follow-up of male hypogonadism as well as female hyperandrogenism. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) is one of the most specific and sensitive technique available in clinical laboratories. The objective of our work was to develop and validate a LC-MS/MS method for testosterone measurement in serum and process to its comparison with immunoassay method.

Materials and methods: The analysis was performed on an Agilent 6460 Triple Quad LC-MS/MS spectrometer with electrospray ionization (ESI). A liquid/liquid extraction (LLE) was used for sample preparation. The calibration curve ranged from 0.025 ng/mL to 50 ng/mL. The method comparison was performed with 80 samples (65 males and 15 females) using the DxI® immunoassay (Beckman Coulter).

Results: The calibration curves showed good linearity with a mean R^2 coefficient of 0.9986 (ANOVA P-value: 2.45E-08). The limit of quantification (LOQ) of the method was set at 0.01 ng/mL. Passing Bablok regression analysis showed slopes of 1.07 and 0.58 in males and females, respectively. Bland Altman plots revealed mean differences of -0.01 and -0.11 ng/mL for males and females, respectively. The method showed accurate results according to external quality control samples (UKNEQAS). The chromatographic conditions also showed a clear separation between testosterone, aldosterone, androstenedione, 17-OH-progesterone and 11-deoxycortisol allowing a simultaneous measurement of those analytes.

Conclusion: Our LC-MS/MS method is sensitive enough to measure testosterone levels in female samples with a good accuracy. The bias observed compared to immunoassays is more important in female than in male samples.

P07-12

Pheochromocytoma biochemical diagnosis

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Background: Pheochromocytoma is an adrenal tumor which produces catecholamines. The measurement of catecholamines and/or its metabolites in blood or urine are essential to diagnosis. It isn't clear from which value the diagnosis should be considered. This work compares levels of catecholamines or its metabolites in urine among patients with and without pheochromocytoma.

Materials and methods: Selection of patients with pheochromocytoma diagnosed from January/1990 to December/2011, followed in Centro Hospitalar do Porto, and patients with false positive tests. Demographical, clinical and analytical data were collected and analysed in SPSS 20.0. Results 22 patients had pheochromocytoma (50% were men), with 47.8 ± 16.6 years-old (17-70). Nineteen patients had elevated normetanephrine or metanephrine (the other 3 patients had high levels of norepinephrine, dopamine or VMA). Twenty patients had at least one increased metabolite 3 times above the upper limit (UL) of the reference range (RR). Nineteen patients (86.4%) had at least one increased metabolite 4 times above the UL of the RR. Among patients without pheochromocytoma, sometimes one metabolite was increased (mostly norepinephrine, normetanephrine or VMA), but it was always below 2.5 times the UL of the RR. In 17 (77%) patients, the metabolites levels weren't above 2 times the UL of the RR.

Conclusions: In the authors' laboratory, the pheochromocytoma biochemical diagnosis must be considered when there is an increased metabolite above 4 times the UL of the RR. When the levels are increased just 2 or 2.5 times the upper limit of the RR, it should be excluded causes of false positive results.

P07-13

Comparison of the results of HPLC methods for determination of methanephrine and normethanephrine from urine and blood plasma considering the diagnosis of tumor pheochromocytoma

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Background: Determination of catecholamines and their O-methyl metabolites normetanephrine (NMN) and metanephrine (MN), in biological fluids plays an important role in the diagnosis of pheochromocytoma (PHEO) - chromaffin cells tumor.

The aim of the study was to compare a HPLC with electrochemical detection method (HPLC-ED) for the determination of methanephrine and normethanephrine in blood plasma and a HPLC with fluorescence detection method (HPLC-FLD) for the determination of the same analytes in conjugated form from urine. Both methods interpret the results in relation to the presence of PHEO. The ability of methods to distinguish the patients with and without PHEO has been proved.

Materials and methods: Determination of metanephrines from plasma (HPLC-ED) and from urine (HPLC-FLD). Standards: NMN ((±) normetanephrine hydrochloride), MN ((±) metanephrine hydrochloride) and HMBA (4-hydroxy-3-methoxybenzylamine-hydrochloride) used as internal standard (Sigma-Aldrich, St.Louis, USA). Chromatography: HPLC system Agilent 1100 (Agilent Technologies, Wilmington, USA). Metanephrines from plasma are determinated in free form, for this reason is possible to directly start by solid phase ex-

traction (SPE) without necessity of hydrolysis. Urine sample is necessary to pass through an acid hydrolysis. During this procedure, releasing of conjugated metanephrines from a bond is happened. Hydrolysis is folloved by SPE. Eluted samples are applied onto a HPLC reversed phase column.

Results: The sensitivity and specificity of methods in tumor diagnosis has been calculated. It has been shown that the sensitivity of both methods has reached 100%, the specificity of methods is lower (94% for the HPLC-ED method and 80% for the HPLC-FLD).

Conclusions: The sensitivity shows excellent ability of both methods to recognize the patients with PHEO. Weaker specificity mainly of the HPLC-FLD method rarely admits false-positive results.

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P08 – Evaluation of analytical systems

P08-01

Evaluation of the Sysmex UF-1000i urinanalyzer

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Background: The aim of this study was to compare the results of Sysmex UF 1000i analyzer for red blood cells (RBC), white blood cells (WBC), epithel cells (EPI), small round cells (SRC) and pathological cast against manual microscopy of uncentrifuged urine specimens using Fuchs-Rosenthal cell counting chamber.

Materials and methods: Sample size: 500 outpatient urines. Carryover, precision, Passing&Bablok regression, Pearson correlation, Receiver Operating Curves (ROC) and diagnostic accuracy were tested.

Results: Carry over: 0.465% for RBC, 0.117% for WBC 0,19%, for EPI and 0.058% for BACT. Within-run imprecision of cell counts expressed as CV% (mean cell count/µl) was found for RBC 6.86%, for WBC 6.97%, for EPI 35%. Between run imprecision was carried out in 30 replicates of two urine control at different concentrations for RBC, WBC, EPI, BACT, and CAST. The mean and CV% was calculated. Passing-Bablok regression were determined for RBC: y = -0.0321 + 1.0383x, for WBC: y = -0.2990 + 1.0499x, for EPI y = 0.2285 + 1.0191x, for SRC y = -0.2161 + 1.6129x, for CAST y = 0.0 + 4.2666x, respectively. Diagnostic accuracy of Sysmex UF1000i showed the following results: 93.2% for RBC, 97.2% for WBC, 92.6% for EPI, 97% for SRC and 60.0% for PC. (Further results: Sensitivity data are: RBC: 96.4%, WBC: 98.0%, EPI: 96.7%, SRC: 60.0%, PC: 79.6%. Specificity data are: RBC: 90.5%, WBC: 95.1%, EPI: 89.7%, SRC: 98.2%, PC: 56.9%.)

Conclusion: Sysmex UF 1000i urine analyzer eliminated manual sample preparation. It has proven good precision for analyzing cellular elements.

P08-02

Evaluating performance of Cobas c311 and Cobas c501 in the emergency laboratory setting

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Background: The aim of our study was to perform the analytical validation of the automated biochemistry analyzers Cobas c311 and c501 (Roche Diagnostics, Germany), for the purposes of the emergency laboratory.

Materials and methods: Validation included assessment of within-day (N = 30) and between-day imprecision (N = 30), inaccuracy (bias) (N = 30), linearity and method comparison with the hitherto used analyzer Olympus AU2700 (N = 30). Statistical