P21-15

HPLC of carbohydrate-deficient transferin and more sialylated transferrin glycoforms in children

Bianchi V (1), Raspagni A (2), Arfini C (3), Vidali M (4)

(1) SSS Antonio e Biagio Hospital, Toxicology Laboratory, Alessandria, Italy
(2) Insubria University, School of Medicine, Varese, Italy
(3) SS Antonio e Biagio, Clinical Laboratory, Alessandria, Italy
(4) Maggiore della Carità Hospital, Clincal Chemistry Unit, Novara, Italy

Corresponding author: vbianchi@ospedale.al.it

Background: The evaluation of age-specific distribution of transferrin glycoforms in paediatric patients may help in defining reference intervals which are critical for an improved and earlier diagnosis.

Materials and methods: Serum samples from 224 children (age: 2 months-14 years) were analyzed by HPLC (CDT by HPLC kit, Bio-Rad, Munich, Germany) and glycoforms expressed as percentage of the total area of transferrin (Tf).

Results: Asialo- and Monosialo Tf were not detectable in any patient. Median (IQR) were respectively 0.92% (O.80-1.04%) for DisialoTf; 3.47% (2.69-4.18%) for Trisialo-Tf; 82.54% (81.32-83.53%) for Tetrasialo-Tf; 12,73% (11.91-14.09%) for Pentasialo-Tf. Statistically significant differences in Trisialo-Tf ($P < 0.001$), Tetrasialo-Tf ($P = 0.001$), Pentasialo-Tf ($P < 0.001$), but not in Disialo-Tf, were observed between the age groups.

Conclusions: Age-specific Diaisialo-Tf cut-offs are not necessary. In children 1.3% and 6.4% may be suggested as upper limits of normal range to detect increases of Disialo- and Trisialo-Tf. The presence of Asialo- and Monosialo-Tf should be considered an abnormal finding and prompt further investigations.

P22 - Vitamin D - PTH

P22-01

Comparison of total 25-OH vitamin D automated immunoassays versus LC-MS/MS

Bausela C, Gaviña B, Ortega I, Torrejón M, Arroyo M

Hospital Clínico San Carlos, Análisis Clínicos, Madrid, Spain

Corresponding author: cris_bausela@hotmail.com

Background: Vitamin D has an important role in both calcium homeostasis and bone metabolism .The best serum marker to determine nutritional status is Total 25-OH Vitamin D. The great methodological variability and the lack of international 25-OH Vitamin D standards for immunoassays, take to wrong classifications, treatments and monitoring. The aim of this study is to compare three automated immunoassay methods for the measurement of Total 25-OH vitamin D (Abbot, Roche and DiaSorin) with LC-MS/MS.

Material and methods: Human serum samples (N = 150) were measured with the followings methodologies:

- DiaSorin LIASION 25-OH VITAMIN D TOTAL CLIA®
- Abbot Diagnostic ARCHITECT 25-OH VITAMIN D CMIA®
- Roche ELECSYS 25-OH VITAMIN D TOTAL ECLIA®
- LC-MS/MS ASSAY

Results obtained by the three immunoassays were compared with LC-MS/MS by linear correlation plots and concordance correlation coefficients. Weight Kappa Coefficient was used to determine the agreement between different assays. All data were analyzed with SPSS software (version 15.0).

Results: Spearman´s Rho Coefficients: DiaSorin = 0.840, Abbot = 0.848 and Roche = 0.817. Intraclass Correlation Coefficients, ICC: DiaSorin = 0.931, Abbot = 0.913 and Roche = 0.897. Weight Kappa Coefficients: DiaSorin = 0.701, Abbot = 0.734, Roche = 0.718.

Conclusions: Correlation and concordance coefficients between the three immunoassays and LC-

MS/MS is good and shows that immunoassays methods are equivalent. However, individual comparison of some data shows that patients can be classified in a different group, according with the method used in the measurement. Therefore, each laboratory should establish their own cutoff points.

P22-02

Stability of PTH in blood specimens after delayed processing

Fernandez Leivas A, Garcia Moreira V, Laborda Gonzalez B, Martinez Gago M, Garcia Alonso S, Fernandez Rodriguez E

Hospital de Cabueñes, Servicio de Analisis Clinicos, Gijon, Spain

Corresponding author: anafleivas@hotmail.com

Background: The parathyroid hormone is secreted by the parathyroid gland (homeostasis of calcium and phosphorus). The recommended preanalytical processing for iPTH is cold centrifugation the sample immediately after collection and frozen until analysis. The aim of our study was to analyze the variability in the results of iPTH comparing two preanalytical protocols.

Materials and methods: 62 serum samples were collected in duplicate and iPTH was measured by electrochemiluminescence immunoassay: autoanalyzer Cobas e411 (Roche). The samples were processed with different preanalitical conditions: immediate centrifugation and freezing (group 1, reference) and sample at room temperature ≥ 2h, centrifugation and frozen until analysis (group 2, study). Significant differences were determined by Student paired-t, using the software MedCalc. Significance was set at $P < 0.05$.

Results: The mean iPTH group1 was 85.07 pg/mL (95%CI: 66.35-103.79) while iPTH in the group2 was 84.54 pg/mL (95%CI: 65.42-103.65). There was no significant difference between groups (paired t-test, $P > 0.05$). The coefficient of variation described by the commercial insert of the method (also verified by our laboratory) is 4.17 and the obtained according to the duplicate samples of the study between two groups was 4.30, so no differences were observed.

Conclusions: According to results, in the iPTH cuantification is unnecessary the immediate centrifugation of the sample. The determination may be made in the same serum tube used to analyze the different biochemical parameters, whose centrifugation is usually done between 2 and 3 hours after collection; this protocol can avoid preanalytical errors, saving time and expenditure rationalization.

P22-03

Evaluation of automated method for measurement of serum 25-hydroxyvitamin D

Vogrinc Ž, Lovrić M, Sertić J

Clinical Hospital Center Zagreb, Clinical Institute of Laboratory Diagnostics, Zagreb, Croatia

Corresponding author: zvogrinc@kbc-zagreb.hr

Background: The most reliable indicator of vitamin D status in organism is measurement of circulating 25-hydroxyvitamin D (25(OH)D) in serum or plasma. The measurement is challenging because 25(OH)D is highly lipophylic, bound strongly to protein, present in low concentrations and exsists in two structurally similar forms, $25(OH)D_3$ and $25(OH)D_2$. The aim of the study was to evaluate a new automated assay for quantitative determination of serum total 25(OH)D (Roche Diagnostics) and compare it with selective and sensitive HPLC method with UV detector.

Materials and methods: 25(OH)D from human sera was measured using two methods: fully automated competitive electrochemiluminiscence method (Roche Elecsys Vitamin D total assay) and Chromsystems HPLC method for 25(OH) D_3/D_2. The evaluation protocol consisted of within-run imprecision (10 sequential runs) and between-run imprecision (10 consecutive working days, 2 sequential runs) with commercial controls PreciControl

Bone 1 and 2, inaccuracy (N = 20), and method comparison (routine serum samples, N = 37). Methods were compared by Passing and Bablok regression and Bland-Altman analyses.

Results: Within-run imprecision for Roche Elecsys Vitamin D total assay was 2.56%, and between-run imprecision was 2.99% and 3.79%. Quality requirement for inaccuracy was fulfilled. The comparison with HPLC method demonstrated strong correlation (r = 0.9581; y=0.918x-4.5082) and good agreement (bias = ± 1.96 SD).

Conclusion: Roche Elecsys Vitamin D total assay showed good correlation and agreement with HPLC-UV method and represents an accurate and precise automated tool for serum total 25(OH)D determination.

P22-04

Vitamin D status in heart failure patients is dependent on the assay method used

Silva N (1), Martins S (2), Tuna D (2), Lourenço P (3), Bettencourt P (4), Guimaraes J (1)

(1) Faculty of Medicine, University of Porto & Sao Joao Hospital, Biochemistry Department & Clinical Pathology Department, Porto, Portugal
(2) Sao Joao Hospital, Clinical Pathology Department, Porto, Portugal
(3) Sao Joao Hospital, Internal Medicine Department, Porto, Portugal
(4) Faculty of Medicine, University of Porto & Sao Joao Hospital, Internal Medicine Department, Porto, Portugal

Corresponding author: jtguimar@med.up.pt

Background: Heart failure (HF) is a prevalent public health problem. Studies indicate a beneficial role for Vitamin D (VTD) on cardiovascular health. Knowing that VTD measurement is highly method dependent, our aim was to evaluate how two different automated assays influence VTD status classification in HF patients.

Materials and methods: Serum samples from 134 patients (65 males and 69 females with 75 ± 13 years old) were evaluated using two chemiluminescent immunoassays (Roche Cobas®- routine method, and Abbott Architect®). Three groups were set: ≤ 10 ng/mL for deficient (A); 11-20 ng/mL for insufficient (B) and > 20 ng/mL for optimal (C). The statistical analysis was performed in Medcalc® software.

Results: For Architect® the values ranged from 5.2 ng/mL to 29.8 ng/mL with a mean value of 14.3 ng/mL. For Cobas® ranged from 3.0 ng/mL to 23.1 ng/mL with a mean value of 8.2 ng/mL. The correlation coefficient was 0.8295, with a mean difference of 6.0 ng/mL, (95%CIs, [5.5; 6.7], P < 0.001). When evaluated on Architect®, 73 (54%) of the patients changed VTD group status: 59 from A to B and 14 from B to C. From the 32 samples which had a value of < 3.0 ng/mL (LOD) on Cobas®, 21 patients continued in A group while 11 changed to B. The mean difference for each group was 6.1, 6.1 and 6.4 ng/mL, respectively.

Conclusion: Pathologists and clinicians must be aware of the clinical consequences that method selection has on patients Vitamin D status classification.

P22-05

Comparison of vitamin D3 and total vitamin D values

Županić D, Crnokrak S, Juričić G, Jedrejčić K, Honović L

Pula General Hospital, Department of Laboratory Diagnostics, Pula, Croatia

Corresponding author: danijela.zupanic@pu.t-com.hr

Introduction: Vitamin D is a fat-soluble vitamin, which is mainly produced in the skin from sun exposure. To become biologically active, vitamin D undergoes two hydroxilations in the liver and kidney. There are two important forms of vitamin D (D_2 and D_3). While vitamin D_3 is produced in the body, D_2 is derived from food and supplements.

Objective: We wanted to show the value of vitamin D_3 and total vitamin D (D_2 and D_3) in the same patients.

Materials and methods: The study included 40 patients (30 women and 10 men). The values of vitamin D_3 and total vitamin D were determined by the immunoassay on analyzer COBAS e601 (Roche, USA).

Results: The study group value of vitamin D_3 < 10 nmol/L was found in 6 patients, 10-30 nmol/L in 10 patients, 30-75 nmol/L in 22 patients and > 75 nmol/L in 2 patients. Total vitamin D was determined for the same patients; the value of total vitamin D < 10 nmol/L was not detected in any patient, 10-30 nmol/L in 5 patients, 30-75 nmol/L in 23 patients and > 75 nmol/L in 11 patients.

Conclusion: Based on the results we can conclude and confirm that the method for total vitamin D determines both vitamin D_3 and vitamin D_2 because the values are higher than those from the method that determines only vitamin D_3.

P22-06

Comparison of two immunoassays for 25-OH vitamin D

Mägi M

East-Tallinn Central Hospital, Central Laboratory, Tallinn, Estonia

Corresponding author: maiga.magi@itk.ee

Background: There are several analytical methods used for measuring the concentration of 25-OH vitamin D in serum. The purpose of this study was to compare the concentrations of vitamin D using two different immunoassays: electrochemiluminescence immunoassay (ECLIA, Roche Cobas e411) and enzyme-linked immunosorbent assay (ELISA, Euroimmun AG).

Material sand methods: First the vitamin D concentrations in serums were measured with ECLIA and according to this results were divided into three groups based on vitamin D recommendations by National Osteoporosis Foundation. First group – vitamin D concentration below 25 nmol/L (50 serums), second group -concentration between 25-75 nmol/L (48 serums) and third group – concentration 75 nmol/L or higher (50 serums). All the serums of three groups were re-measured with both methods in one batch at the same day.

Results: The coefficient of determination (R^2) was 0.577 ($P < 0.001$) for the first, 0.859 ($P < 0.001$) for the second and 0.669 ($P < 0.001$) for the third group. The linear regression between methods was ELISA (y) = 1.28*ECLIA (x) + 24.66 (SE = 5.59) for the first, ELISA(y) = 0.94*ECLIA (x) + 17.73 (SE = 5.96) for the second and ELISA (y) = 0.89*ECLIA (x) + 25.46 (SE = 11.15) for the third group.

Conclusions: There was no good correlation between two methods used in this study. The correlation between two assays was best for the second group containing serums with 25-OH vitamin D concentrations between 25-75 nmol/L. Additional studies containing more methods are required to evaluate and compare the methods used for measurement of 25-OH vitamin D.

P22-07

25-hydroxi-vitamin-D levels in clinical conditions with low plasma albumin

Kovacs G (1), Horvath D (2), Viragh E (2), Kovacs L (3), Locsei Z (2), Toldy E (4)

(1) University of Pecs, Institute of Laboratory Medicine, Pecs, Hungary
(2) Markusovszky Teaching Hospital, 1st Department of Medicine, Szombathely, Hungary
(3) Markusovszky Teaching Hospital, B. Braun Avitum Dialysis Centre, Szombathely, Hungary
(4) University of Pecs, Institute of Diagnostics, Szombathely, Hungary

Corresponding author: gabor.l.kovacs@aok.pte.hu

Introduction: Majority of circulating total 25(OH) D is bound to proteins. 90% is bound to vitamin D binding protein (DBP), an alfa-2globulin (a-2-Gl)

fraction, and 10% to albumin (ALB). Nowadays the total t25(OH)D level is considered to be the most widely accepted marker of vitamin D supply in physiological states. Under pathological circumstances (e.g. in diseases with low ALB), however, the situation might be different. Our aim was to investigate the t25(OH)D concentration in clinical conditions with low ALB levels.

Materials and methods: 95 patients (48 men, 47 women; mean age: 68.2 ± 14.4 years) with low ALB (31.9 ± 5.9 g/L) were studied. 58 patients had chronic renal failure, 8 nephrosis, 17 cirrhosis and 12 malnutrition. 58 healthy adults (30 men, 28 women; mean age: 65.9±15.8 years) were in the control group. 25(OH)D, intact parathormon (PTHi), calcium, TP, ALB, DBG, a-2-Gl were measured.

Results: 90% of the patients with low ALB had vitamin D deficiency (< 50 nmol/L). Low vitamin D, however, also occurs among healthy people. Vitamin D deficiency is much more frequent in cases with low DBP (< 272 mg/L), than in those with normal DBP. There was a correlation between vitamin D and DBP. This was much stronger in the group of patients with renal failure.

Conclusion: Our results suggest that in clinical conditions with low ALB levels - especially if hypalbuminaemia is associated with low DBP and excess of a-2-Gl - t25(OH)D levels may not only depend on vitamin D supply, but also on the presence and capacity of the binding proteins.

P22-08

Intraoperative parathyroid hormon measurements in femal with parathyroid adenoma

Mayer L (1), Gaće M (1), Dobrijević S (1), Špacir-Prskalo Z (1), Janušić R (2), Ramljak V (3)

(1) Sestre Milosrdnice University Hospital, University Hospital for Tumors, Clinical Institute of Chemistry, Zagreb, Croatia
(2) Sestre Milosrdnice University Hospital, University Hospital for Tumors, Department of Head and Neck Surgery, Zagreb, Croatia
(3) Sestre Milosrdnice University Hospital, University Hospital for Tumors, Department of Cytology, Zagreb, Croatia

Corresponding author: ljiljana.mayer@kbcsm.hr

Primary hyperparathyroidism (PHPT) is disorder characterized by increased and uncontrolled parathyroid hormone secretion, cause of hyperfunction of one or more parathyroid glands. In 80-85% cases of PHPT is caused by parathyroid adenoma. Persistent hyperparathyroidism leads to altered osseous metabolism involving bone resorption and tissue changes. In rare cases, approximately in every thirteenth patient with PHPT, the bone mass is suspected of being a neoplastic lesion - brown tumor induced by primary hyperparathyroidism. The only way of PHPT correction is surgical elimination hyperactive parathyroid glands. In this article we report the first case of intraoperative parathyroid hormone measurements for primary hyperparathyroidism in Republic of Croatia. The possibility of intraoperative PTH monitoring provides an additional patient and operator safety. Intraoperative PTH becomes an exact instructor (navigator) to operator –surgeon. On the basis of decreasing value of this peptide with very short half-life time, surgeon makes immediate decision if the operation is completed or he requires further excision parathyroid glands because of hyperplasia. Guarantee of successful diagnosis, which is a prerequisite for the correct treatment, is a multidisciplinary, continuous, systematic and synchronized cooperation of whole and heterogeneous medical team, which includes clinicians, radiologists, cytologists, pathologists and medical biochemists.

P22-09

Patients with colorectal cancer have profound deficiency of vitamin D

Greplova K (1), Valik D (1), Obermannova R (2)

(1) Masaryk Memorial Cancer Institute, Department of Laboratory Medicine, Brno, Czech Republic
(2) Masaryk Memorial Cancer Institute, Department of Comprehensive Cancer Care, Brno, Czech Republic

Corresponding author: greplova@mou.cz

Background: Vitamin D plays an important role in a number of physiological functions including calcium absorption, bone metabolism, immune function, muscle function and cellular regulation. Numerous clinical studies have shown that vitamin D has significant protective effect against the development of cancer. We studied changes in 25(OH)D serum concentrations at different temperature storage conditions. Subsequently we measured 25(OH)D serum concentration in 100 healthy individuals and 281 patients with colorectal cancer.

Materials and methods: Blood samples were taken in sample tube without anticoagulant (Sarstedt, catalog number 01.1728.001). After centrifugation (1500 x g, 15˚C, 20 min), separated serum was pipetted into 3 test tubes. 25(OH)D was measured 1) immediately after serum separation 2) after 3 weeks of freezing at -80 ˚C 3) after 3 weeks of freezing at -30˚C. 25(OH)D serum concentrations were measured using Architect i2000sr (Abbott) analyzer.

Results: We didn't find any significant concentration changes of 25(OH)D in 100 samples at various temperature storage conditions. In the group of healthy individuals median of 25(OH)D concentration was 55.35 nmol/L (range within 21.7-116.4 nmol/L), yielding reference range 30-90 nmol/L (95% confidence interval). In the group of colorectal cancer patients median of 25(OH)D was 26.1 nmol/L (range within 0-84.7 nmol/L).

Conclusions: The CMIA method proved to be robust and stable for clinical determination of serum 25(OH)D levels. In colorectal cancer patients we observed profound deficiency of vitamin D.

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P22-10

Vitamin D mediated inhibition of cancer: Do cytokines play a role?

Mohapatra S (1), Saxena A (1), Gandhi G (2), Singh T (3), Koner BC (1), Ray PC (1)

(1) Maulana Azad Medical College, Department of Biochemistry, New Delhi, India
(2) Maulana Azad Medical College, Department of Obstetrics and Gynecology, New Delhi, India
(3) Maulana Azad Medical College, Department of Pathology, New Delhi, India

Corresponding author: sudhesna.moh@gmail.co

Background: There exists an inverse relationship between vitamin D levels in blood and incidence of many cancers. Vitamin D suppresses pro-inflammatory Th1 cytokines (TNF-α) and promotes Th2 subtype differentiation (as marked by rise in IL-4). In ovarian cancer, the tumour microenvironment is enriched with a broad spectrum of pro-inflammatory cytokines which helps in tumour progression. Role of vitamin D in ovarian cancer has not yet been clearly defined.

Materials and methods: A case control study was conducted recruiting fifty ovarian cancer patients and fifty controls. Serum vitamin D, TNF-α and IL-4 were measured in fasting blood sample of the subjects.

Results and conclusions: Serum vitamin D levels were significantly ($P < 0.033$) lower in ovarian cancer cases [20.1 ng/mL (61.8-6.93)] as compared to controls [4.6 ng/mL (47-7.3)] which was more evident in post-menopausal group of ovarian cancer patients. TNF-α levels were significantly higher in ovarian cancer patients [cases: 12.2 pg/mL (21.0-

5.1); controls: 6.2 pg/mL (12.0-2.0); P < 0.001] and IL-4 levels were significantly lower as compared to those of controls (cases: 2.22 ± 0.51 pg/mL; controls: 2.99 ± 0.68 pg/mL; P < 0.001). Vitamin D levels were negatively correlated (R^2 = 0.092, P < 0.034) with TNF-α and positively correlated (R^2 = 0.227, P < 0.001) with IL-4. None of the ovarian cancer patients had serum vitamin D level in highest tertile of the study group. Our study provides evidence that increased Th1 cytokines release and decreased Th2 response are associated with low serum vitamin D which might be a risk factor for ovarian cancer. This indicates that supplementing vitamin D might be protective against ovarian cancer (especially in postmenopausal women) by modulating the cytokine environment.