Abstracts*)

10th National Congress of the Portuguese Society of Clinical Chemistry, Genetics and Laboratory Medicine

Place:
Figueira da Foz, Portugal

Date:
13 - 14 April 2018

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### P01

**RELEVANCE OF THE THERAPEUTIC MYCOPHENOLIC ACID MONITORING IN KIDNEY TRANSPLANT**

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**Introduction:** Mycophenolic acid (MPA) is widely used as an antirejection drug after renal transplantation. There is growing evidence supporting the notion that there is substantial variability in the intra and interpatient exposure to MPA. The aim of this study was to determine if MPA through concentrations in kidney transplant recipients are in therapeutic levels and clarify if therapeutic drug monitoring of MPA should be considered.

**Materials and Methods:** We conducted a cross-sectional study enrolling 58 kidney transplant recipients with more than 6 months of follow-up, receiving MPA in a steady dose (500 + 500 mg). Clinical and laboratory data including anthropometric data and glomerular filtration rate (eGFR) were obtained from patients’ electronic charts. Individual MPA trough concentrations were accessed using high-performance liquid chromatography (Agilent®, 1100 series), and were classified into sub-therapeutic (≤ 1.9 mg/L), therapeutic (> 1.9 mg/L, < 12 mg/L) and toxic (≥ 12 mg/L). MPA trough concentrations were evaluated in relationship to covariates age, body weight and eGFR. Statistical analysis was performed using Stata 14.0.

**Results:** In our sample of 58 patients, male patients represented 60.3% (35) and the mean age was 52.4 ± 11.2 years old. The median kidney graft follow-up was 6.3 (0.6, 19.2) years and 100% of the patients were receiving tracolimus. The median MPA trough level was 2.1 mg/L (interquartile range 1.5 - 2.2). Despite that, 43.1% (25) exhibit sub-therapeutic levels, 51.7% (30) therapeutic levels and 5.2% (3) toxic levels. Age was negatively (and significantly) correlated with lower MPA trough concentrations (coefficient -0.28, p < 0.035). No relationship between MPA trough concentrations and weight and eGFR were found.

**Conclusion:** Our study strongly supports the need of therapeutic drug monitoring of MPA therapy to avoid underimmunosuppression and toxicity. We identified age as a risk factor for MPA sub-therapeutic levels, with the oldest patients being the most affected. This might be linked to the fact that older patients are often polymedicated and therefore, with higher risk of drug interaction. For this reason MPA concentrations should be monitored carefully and dose adjusted whenever MPA concentrations are not in therapeutic levels.


### P02

**SEXUAL DIMORPHISM IN FIRST TRIMESTER PLACENTAL ANGIogenic MARKERS: REPERCUSSIONS FOR PREECLAMPSIA RISK PREDICTION MODELS**

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**Introduction:** Previous studies suggest that the fetoplacental unit affects physiological and pathologic mechanisms of pregnancy in a sex specific manner. We aimed to assess the influence of fetal sex on the prognostic value of first trimester placental angiogenic markers for early prediction of preeclampsia.

**Material and Methods:** Case-control study of 500 participants from a prospective population-based cohort of 10039 singleton pregnancies that underwent routine first trimester aneuploidy screening in a routine care low-risk setting. The study sample included 250 women who subsequently developed preeclampsia and 250 healthy women matched for gestational age at time of blood sampling. First trimester serum placental growth factor (PIGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured by automated electrochemiluminescence assay according to manufacturers’ recommendations (Roche Diagnostics).

**Results:** Women carrying female (50.5%) or male (49.5%) fetuses did not significantly differ concerning first trimester clinical characteristics. Concentrations of sFlt-1 and sFlt-1/PIGF ratio were significantly higher (P < 0.01 and P < 0.05, respectively) in the presence of a female fetus, considering both the preeclamptic group and the whole study sample. Receiver operating curve (ROC) analysis revealed a superior performance for PE prediction by placental angiogenic markers in female pregnancies (P < 0.05).
Conclusion: We found fetal sex related differences in first trimester placental angiogenic markers; such sexual dimorphism effect was particularly strong in preeclamptic pregnancies. Fetal sex should be considered for inclusion in first-trimester prediction algorithms for preeclampsia that integrate placental angiogenic markers.

P03

JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML) – CASE REPORT

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Introduction
Juvenile Myelomonocytic Leukemia (JMML) is a rare and aggressive myelodysplastic/myeloproliferative neoplasm that represents 2-3% of all leukemia cases at pediatric age. It’s usually caused by mutation of genes within RAS/MAPK pathway (NF1, PTPN11 KRAS, NRAS,…) and is characterized by proliferation of myeloid and monocytic lineages, anemia and thrombocytopenia. Diagnosis is made using 2016 World Health Organization criteria. Allogeneic hematopoietic cell transplantation (HCT) is the only potentially curative treatment.

Materials and methods
We will describe a case of JMML in a child with Neurofibromatosis type 1 (NF1).

Results
A 2-year-old boy with NF1 was admitted in pediatrics routine consulting. Although there were no significant changes on physical examination he “looked sick” to his doctor. Laboratory tests revealed: hemoglobin (Hb): 12, 8 g/dL, white blood cells (WBC) 23,470/μL, monocytes 3660/μL, immature cells: 940/μL. In the following study there was an increase of leukocytosis (WBC: 57,250/μL), with “monocyte-like” cells: 14,310/μL. Suspecting of Acute Leukemia, patient was transferred to Instituto Português de Oncologia Porto (IPO).

Virus serological and molecular surveys were negative. Peripheral blood (PB) smear revealed cells of all lineages and two types of monocytes. The flow cytometric analysis of Bone marrow (BM) showed increase of classical monocytes. BM aspiration revealed a hypercellular BM with dysplastic megakaryocytes and hyperplasia of granulocytic and monocytic lineages. BCR-ABL fusion gene was absent. Abdominal ultrasound revealed hepatomegaly, without splenomegaly.

Conclusions
The following results led us to the JMML diagnosis: PB monocytosis > 1×10⁹/L, <20% blasts in PB or BM, absence of BCR-ABL fusion gene and clinical diagnosis of NF1. Patient is waiting for compatible donor for allogeneic HCT.

Due to the rareness and unspecific characteristics of JMML, frequent mistakes and a delay in diagnosis occur. NF1 significantly raises its risk. JMML may mimic or manifest itself after a viral infection so is important to exclude an infection and integrate all clinical and laboratory information (morphology, histology, genetics, and flow cytometry) in order to ensure a reliable diagnosis.

References
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P04

IMMUNOGLOBULIN D GAMMOPATHY – A RARE ENTITY WITH DISTINGUISHING FEATURES

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Introduction: In monoclonal gammopathies, immunoglobulin(Ig)G, IgA and light chains predominate. The IgD type is much less frequent, accounting for 2% of all multiple myelomas (MM) and being exceedingly rare in monoclonal gammopathy of undetermined significance (MGUS).
Methods: Sixteen cases (11\text{\%}, 5\text{\%}) diagnosed heavy chain IgD gammopathy at Coimbra Hospital and University Centre from Jan/2013 to Jan/2018 for were included in this study.

Results: There was a male prevalence, median age was 67.8y (range 45-82y). 25% of the patients were under 60y. Anaemia was present in 73.3% of the cases, hypercalcemia in 26.8% and creatinine was elevated in 48.7% of the patients (x = 2.02 mg/dL). LDH was over reference values in 38.4% of patients. β2-microglobulin was elevated in 91.7% of patients, correlating positively with creatinine levels (p < 0.01).

All but one of the patients showed at least one spike on electrophoresis (93.3%), with 1/3 displaying 2 spikes. The monoclonal proteins migrated along the β and γ regions. A λ light chain bias was noticed in 93.3% of the patients. Reversed κ/λ ratio was found in 100% of patients. Elevated values of IgD were confirmed in all patients. Higher λ free light chain (FLC) were associated with higher creatinine levels (p < 0.01) and higher β2-microglobulin values (p < 0.01).

Urinary FLC were reported in 87.5% of tested patients. Patients were mainly MM (79\text{\%}), followed by plasma cell leukaemia (14\text{\%}) and MGUS (7\text{\%}). Survival was studied for this population. The highest death rate was in the first 6 months after diagnosis, with 4 patients dying. Remarkably, higher LDH levels were found to be associated with reduced survival (p < 0.05). The survival rate at 2 years was 66.7\text{\%}.

Conclusion: Patients with IgD gammopathy presented often with features of high-risk disease such as renal dysfunction and urinary FLC. Predominance of λ light chain expression with reversed κ/λ ratio was consistent with the literature.

Higher λ FLC seemed to be related to worse renal function, which can cause an increase of β2-microglobulin. According to our results, elevated LDH value could be predictive of worse prognosis. No further correlations with the prognosis were found. The role of laboratory analysis remains pivotal in detecting and fully characterizing gammopathies. More studies are needed to better characterize IgD gammopathies.

References:

P05

ABNORMALITIES OF COAGULATION IN PATIENTS WITH DECREASED GLOMERULAR FILTRATION RATE

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Introduction: Previous studies reported that patients with chronic kidney disease (CKD) have blood coagulation disorders. An increased bleeding tendency was reported in 40–50\text{\%} of patients with chronic renal failure or on haemodialysis (HD). Considering these studies we aimed to evaluate the prothrombin time (PT) and activated partial thromboplastin time (aPTT) in patients with a decreased glomerular filtration rate (GFR).

Material and methods: The results from two years were collected anonymously from hospital laboratory middleware database. Along with the laboratory parameters, age, sex and diagnosis were also collected. The GFR was estimated from serum creatinine according the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. According the GFR, patients were organized in five groups: Group 1: GFR >90 mL/min/1.73 m²; Group 2: GFR 60-89 mL/min/1.73 m²; Group 3: GFR 30-59 mL/min/1.73 m²; Group 4: GFR 15-29 mL/min/1.73 m² and Group 5: GFR <15 mL/min/1.73 m². The study was limited to outpatient clinic adults without anticoagulant therapy (except patients from group 5).

The reference interval for PT was 12.0-17.4s and for aPTT 25-34s.

Results: 217 patients were enrolled in this evaluation with a mean age of 56 ± 20 years old. Considering the GFR, 38.2\text{\%} of the patients were in group 1, (PT = 15.6 ± 4.8s; aPTT = 29.53 ± 10.3s); 18.4\text{\%} in group 2, (PT = 17.0 ± 6.8s; aPTT = 27.9 ± 4.1s), 24.9\text{\%} in group 3, (PT = 21.1 ± 9.5s; aPTT = 31.5 ± 7.6s); 12.3\% in group 4, (PT = 23.9 ± 7s; aPTT = 35.6 ± 13.8s); and 4.1\% in group 5, (PT = 27.7 ± 12s; aPTT = 38.6 ± 11.9s). Patients from groups 3-5 were characterized by a significantly increase in the PT and aPTT as compared to that in groups 1, 2, (P < 0.05). The analysis of the correlation between GFR and the PT and aPTT showed a statistically significant positive coefficient.

Conclusions: Patients with a GFR below 59 mL/min/1.73 m² have a significant increase of the PT and aPTT as compared to that in group 1 and 2. This increase was particularly significant in patients with GFR < 30 mL/min/1.73 m². In group 5 the prolonged aPTT could be associated to the regular heparin usage during conventional HD. In group 4 and 3 the increase in PT and aPTT is probably associated to the fact that the decrease in GFR is associated to the existence of chronic, slowly progressing and systemic diseases.

P06

ALGORITHM DEVELOPMENT FOR THE IMPLEMENTATION OF HIGH SENSITIVITY CARDIAC TROPINON I MEASUREMENT IN A TERTIARY HOSPITAL

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Introduction: Chest pain is one of the most common symptoms in patients attending the emergency department, making its adequate evaluation of utmost importance. The diagnosis of non-ST segment MI depends on the early detection of cardiac troponin (cTn) and new assays of high sensitivity cTn (hs-cTn) have been recently introduced, with the potential to alter patient management.

Material and Methods: Review of the development and pre-implementation of a diagnostic algorithm for the use of hs-cTn in patients with chest pain. This algorithm was developed by the clinical pathology department in collaboration with the cardiology and emergency departments and implemented on the 5th of March 2018.

Results: For optimal use of the hs-cTn assay a diagnostic algorithm for both rule-in and rule-out of MI was developed. The importance of dynamic changes in hs-cTn serial testing as well as cut-off values according to gender were taken into account. These decisions were made in line with the latest European Society of Cardiology guidelines, with several validated studies as well as considering the reality of our hospital. The resulting algorithm includes a rule-out value of ≤5ng/mL if chest pain >3 hours and rule-in value if hs-cTn is 10x higher than the 99th centile. For patients with values >99th centile and for patients with values <99th centile but with chest pain <6 hours, serial testing at presentation and at 3 hours is recommended, with a change of >50% at 3 hours highly suggestive MI. For patients with values <99th centile and chest pain >6 hours other causes need to be investigated. For educational purposes presentations aimed at the clinical staff were performed.

Conclusion: The use of hs-cTn assays in clinical practice has many potential advantages as long as a comprehensive algorithm is developed, and an educational process is tied to its introduction. While earlier rule-out is seen as one of the major advantages of hs-cTn assays, a rule-in capable of accurate identification of patients with MI is also important as both approaches can potentially reduce costs and improve outcomes. It’s also critical to evaluate dynamic changes with serial testing and not interpret hs-cTn as a binary assay. Although not yet in clinical practice, our algorithm includes a rule-out cut-off, not widespread in our country, that we aim to evaluate in the future.

References:

P07

A NOVEL FLOW CYTOMETRIC PROTOCOL FOR THE EVALUATION OF COLISTIN ACTIVITY

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Background: Colistin (CS) constitutes the last resort for infections caused by multidrug-resistant Gram-negative bacteria. Classical susceptibility tests are laborious, time-consuming and some technical issues have been reported.3

Aims: Optimization of a novel flow cytometric protocol to evaluate qualitative and quantitatively cell physiological changes induced by CS exposure.

Methods: Were included 2 susceptible (P. aeruginosa ATCC 27853 and E.coli ATCC 25922) and 1 resistant strain to CS [E. coli NCTC 13846 (mcr-1-positive, MIC 8 μg/mL)]. Bacteria were inoculated in Muller-Hinton broth (Sigma) until exponential growth phase (± 2 hours). A cell suspension containing 1x10⁶ cells/mL was incubated during 15, 30 and 60 min, at 37 °C with CS (C4461, Sigma) at concentrations ranging between 0.25 to 8 μg/mL, and stained with different fluorescent dyes. Changes in membrane lesion, membrane potential, metabolic activity and ROS production were explored. Afterwards, cells were analyzed in a BD Accuri™ C6 Plus Flow Cytometer. For each tested conditions the intensity of fluorescence of treated cells and non-treated cells were compared determining a staining index. Additionally, this protocol was applied in pure colonies directly from culture plate. Different statistical tests were used (Wilcoxon and Kruskal-Wallis tests). P-value <0.05 was considered significant.

Results: CS induced cellular alterations in susceptibility bacteria, regarding membrane lesion, membrane potential, metabolic activity and ROS production up to 1 hour of exposure. 15 min were sufficient to produce changes in membrane lesion, being this effect dose-dependent. Conversely, in R strain, the studied cellular parameters did not suffer significant changes up to 60 min of incubation. Significant differences were found between S and R strains (p < 0.001). Data obtained for the protocol with pure colonies directly from culture plate were similar to
described above, with the advantage of providing a result in lower time. Moreover, cytometric data showed a high agreement (1.00) with the MIC values of CS previously determined according to CLSI and EUCAST protocols.

**Conclusions:** We hereby presented a new and fast flow cytometric protocol which revealed to be an excellent tool for evaluation of CS activity providing a MIC value in useful time.

**References:**

**P08**

**METHOXYTYRAMINE, QUANTIFICATION BY LC-MS/MS IN PLASMA SAMPLES: MORE WITH THE SAME**

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**Introduction:** Paragangliomas (PGL) are neuroendocrine tumors that arise from the extraadrenal autonomic paraganglia and have the ability to secrete catecholamines. PGL often present clinically like pheochromocytoma (FEO) with hypertension, episodic headache, sweating, and tachycardia. However, due to the risk for malignancy, and genetic testing the distinction between FEO and PGL is important. The recommended screening test for initial assessment of PGLs is the measurement of plasma normetanephrins (NM) and metanephrines (MET). Recent studies showed that the plasmatic 3-methoxytyramine (3-MT), a O-methylated dopamine metabolite, could be important to identify PGL tumors that exclusively secrete dopamine, as well as those that are malignant.

This work aims to improve the already existent measurement of MET and NM implementing simultaneous measurement of 3-MT in the same run, by liquid chromatography tandem-mass spectrometry (LC-MS / MS) in order to contribute to a better diagnosis and monitoring of PGL patients.

**Methods:** After a solid phase extraction procedure of 1 ml of plasma, to extract the analytes and remove plasma interferences, 50 μl of the eluate are injected into the LC/MS-MS. Chromatographic separation was accomplished with an INERTSIL Amide 5 μ, 2.1x250 mm analytical column, calibrators and controls were purchased from Recipe Chemicals (ref. MS11013, ref. MS11080). A Waters Alliance 2695/micromass Quattromicro was used. A convenient gradient mobile phases system with positive electrospray ionization detection was used and specific transitions m/z in the multiple reaction monitoring mode were 151>119 for 3-MT and 155>95 for deuterated.

**Results:** This method is fast, and showed a good profitability since it allows the simultaneous measurement of NM, MET and 3-MT. Reproducibility for all clinical decision level (CV < 7%) and the linearity displayed in all calibration curves (r² > 0.992154) were excellent.

**Conclusion:** LC-MS/MS method proved to be specific, accurate and sensitive to quantify a low nanomolar concentration of 3MT, MET and NM simultaneously. With these measurements a better characterization of the tumour is possible as well as the follow-up of these patients.

**References:**

**P09**

**PHADIATOP™ CASUISTIC IN A PORTUGUESE TERTIARY CARE CENTER**

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**Introduction:** *Phadiatop™* is a commercially available serological screening test of allergic sensitization in patients with suspected allergic diseases. The aim of the study was to assess the casuistic of *Phadiatop™* test results in a tertiary care center population.

**Materials and Methods:** *Phadiatop™* test was performed in serum samples from a total of 1709 patients with suspected allergic disease. *Phadiatop™* positive tests were followed by measurement of specific IgE in serum for respiratory allergens (11 different allergens: d1, d2, e1, e5, g2, g5, m3, m6, t16, w21 and i6). Data, relating to the year of 2017, were collected using *Clinidata XXI* - Maxdata® (LIS), and sorted by sex [male (M): 54,65%; female (F): 45,35%] and age groups [group I (G I): 0-10 yrs; group II (G II): 11-20 yrs; group III (G III): 21-30 yrs and group IV (G IV): >30 yrs].

**Results:** *Phadiatop™* test was positive in 43,18% patients (50,75% F and 34,06% M). In G I (366 pts), 71,04% were male. The most common allergens were *D. pteronyssinus* (d1) (83,61%) and *D. farinae* (d2) (66,67%). In G II 61,89% were M, with the most common allergens being d1 and d2, as well. G III, the group with less patients had a total of 18, being mostly female (66,67%). Mites were clearly the dominant allergens,
followed by grass pollen. G IV had a total of 46 patients, being 60.87% female. Prevalent allergens in this group were d1 and d2 (67.39% and 65.22% positive, respectively), followed by g2 and g5 (43.48% tested positive for both).

**Conclusions:** Near half of Phadiatop™ tests were positive, being more frequent in males of younger age, and in females from the age of 21 and above. The most frequent respiratory allergens – mites and grass pollen – were transversal to all age groups.

**REFERENCES:**

**P10**

**PANCYTOPENIA IN CHILDHOOD AS A MEDICAL CHALLENGE: A CASE REPORT**

**Teresa Ribeiro, Cláudia Reynolds, Lino Azevedo, Cristina Silva, Gabriela Martins; Marta Almeida; Armando Pinto; Teresa Sousa; Carlos Mendes**

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**Introduction:** New-onset pancytopenia in childhood can be caused by a variety of underlying diseases, including hematological and non-hematological, acquired or inherited entities, leading to a diagnostic challenge. Thus, bone marrow examination is often required for an accurate diagnosis.

**Case Report:**
A 2-year-old caucasian boy, with history of fetal anemia transfusion presented with diffuse abdominal pain, fever and scattered petechiae. Blood analysis revealed a new-onset pancytopenia (hemoglobin 7.8g / dL, leukocytes 3420 / uL, neutrophils 570 / uL, platelets 13,000 / uL). Biochemistry, coagulation study, abdominal ultrasound and chest X-ray had no alterations; No microorganisms were isolated in UC and HC; Wide virological and immunological studies were negative. By unexplained persistent pancytopenia, he was transferred to our institution for further investigation. Two consecutive medullary evaluations, in a 3-week period, were carried out. It was revealed hypocellularity and discrete dysplastic changes, with morphological erythroid dysplasia in both, and presence of myeloid dysplasia by immunophenotyping only in the second evaluation; without blasts and without cytogenetic alterations (20 metaphases analyzed showed normal male karyotype). It was performed bilateral bone biopsy confirming hypocellularity, without immature cells (CD34-) being identified.
After exclusion of the main causes, such as infection, acquired marrow failure syndromes and marrow space infiltrating lesions, patient was referred to a specialized center for further investigation, namely congenital bone marrow failure syndromes.

**Conclusion:** Even though congenital bone marrow failure syndromes are rare, these diseases should be considered as a potential cause of new-onset pancytopenia in children because they have serious clinical and treatment implications. That is why, it is still a great challenge to find the correct diagnosis for patients with pancytopenia especially in the presence of a hypocellular bone marrow or in the absence of obvious neoplasm.

**References:**

**P11**

**AEROCOCCUS URINAE – AN USUAL AGENT FOR A COMMON INFECTION**

**Joana Sevilha, Cláudia Reynolds, Catarina Ferreira, Paula Costa, Mariana Viana, Marília Dias**

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**Introduction:** Aerococcus urinae is a rare UTI agent belonging to the Aerococaceae family. It is a Gram positive coccus, α-hemolytic, catalase negative and nutritionally demanding. This microorganism can cause severe complications such as, sepsis, endocarditis and osteomyelitis.

**Material and Methods:** Male patient, 90 years old, brought to the emergency room (ER) due to prostration, asthenia, cough, back pain, dysuria and polyakuria. At the ER admission, he was conscious and had consistent urinary complaints. The urinalysis showed positive leukocyte esterase and negative nitrites. As it was suspected of a sepsis with an urinary / respiratory starting point, inpatient treatment was preferred. Two urine cultures and blood cultures were requested. The urine was seeded by semi-quantitative method on ChromoIDTMCPS®Elite agarose media and blood cultures in automated system Bactec.

**Results:** The microscopic observation of the Gram stain smear, showed intra-leukocytes Gram-positive cocci. After 48 h of incubation, a >100,000CFU/mL of a single type of catalase negative colonies was obtained. Blood agar media showed α-hemolytic colonies. Blood cultures were sterile at 5 days of incubation, and lasted up to 30 days without bacterial growth. The bacterial identification conducted by the automatic method: ID GP-VITEK®2 CompactIdentified Aerococcus urinae. As for the antimicrobial susceptibility test, this bacterium was sensitive to penicillin.
Conclusion: Given the morphological similarities of *Streptococcus spp* and *Enterococcus spp* colonies with *A. urinae* colonies, misclassification and clinical relevance underestimation are frequent. Due to the fact that intra-leukocyte Gram-positive cocci were observed in both urine cultures, the study and valuation of this agent as the causative microorganism of the patient’s complaints were compromised. The patient died 4 days after sampling collection, making it impossible to study the evolution after therapy, as well as the research of possible complications.

References:

**P12**

THE IMPORTANCE OF THE PERIPHERAL BLOOD FILM IN MYELOPEROXIDASE DEFICIENCY IDENTIFY BY AN AUTOMATIZED HEMATOLOGICAL ANALYZER

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Introduction: The hematologic evaluation of the peripheral blood plays an important role in the clinical laboratory daily routine. Nowadays the automated hematological analyzers develop an irreplaceable role for a quick, cheap and reliable performance of the full blood cells count. The myeloperoxidase (MPO) is an enzyme produced by neutrophils, found in azurophilic granules and released upon its activation. The myeloperoxidase deficiency may occur as an inherited or acquired disorder, and is occasionally associated with an increase in susceptibility to certain infections. An association between this deficiency and a vulnerability to certain malignancies has been proposed. Some hematological analyzers identify neutrophils, measuring not only its lobularity/nuclear density but also its MPO content, triggering a flag when the two parameters differ significantly and a myeloperoxidase deficiency is identify.

Material and Methods: We performed a retrospective study (data from January 2015 to February 2018) taking in account the full blood count of blood samples collected in EDTA containers, triggered with a flag of myeloperoxidase deficiency by a automated hematological analyzer, with the characteristics described above. In these full blood counts the results of the differential leukocytes count obtained by the automated hematological analyzer were compared with the results obtained by the observation of a blood film, stained with May-Grünwald Giemsa.

Results: A total of 471 full blood counts were triggered with a flag of myeloperoxidase deficiency by the equipment, for all of them a blood film and the differential leukocytes count were performed. Of those 95.75% presented a different differential leukocytes count between the automated hematological analyzer and the blood film, with a usual larger count of neutrophils in the blood film. In the remaining full blood counts the differential leukocytes counts were superposable.

Conclusions: When in a full blood count a flag of myeloperoxidase deficiency is triggered by a automated hematological analyzer with the characteristics described, a blood film must be performed to evaluate the correct differential leukocytes count.

References

**P13**

SERUM RETINOL AND α-TOCOPHEROL IN THE INFLAMMATORY BOWEL DISEASE

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Introduction: Inflammatory bowel diseases (IBD) are autoimmune and chronic diseases of unknown etiology. IBD comprises Cronh’s disease (CD) and ulcerative colitis (UC) and usually are linked with fat-soluble vitamins deficiency. A cross-sectional study was undertaken to determine the prevalence of low serum retinol and α-tocopherol levels in a non-supplemented population with IBD.

Material and Methods: Clinical information and serum fat-soluble vitamin levels were gathered from 142 patients, 119 with CD and 23 patients with UC, enrolled for the treatment of IBD at São João Hospital Centre. Serum retinol and α-tocopherol levels were determined by high-performance liquid chromatography. Reference values used for serum retinol and α-tocopherol were 30-80 μL.dL⁻¹ and 500-1800 μL.dL⁻¹, respectively.

Results: The mean age of the patients was 41.6 ± 15.1 years old with 80 (56.3%) females. The mean results for serum retinol and α-tocopherol were 50.6 ± 18.8 μL.dL⁻¹ and 1197.3 ± 332.5 μL.dL⁻¹, respectively. There were no significant sex-differences for both serum assays (p > 0.05) neither for the subjects aged below or above 41 years old for serum retinol (p = 0.47). The subjects above 41 years old had significant higher levels of α-tocopherol (+130.8 μL.dL⁻¹; p = 0.02) A strong positive correlation was reported between retinol and α-tocopherol (r² = 0.42). 70.9% (n = 10) of...
the subjects had low serum retinol levels, corresponding to 6.7% (n = 8) of the patients with CD and 8.7% (n = 2) with UC. 0.7% (n = 1) of the subjects had low α-tocopherol levels, corresponding to 0.8% (n = 1) of the patients with CD. No significant differences in serum retinol and α-tocopherol were observed (p > 0.05) regardless the IBD being CD or UC.

**Conclusion:** Some studies reported low serum fat-soluble vitamins levels in IBD, namely retinol and α-tocopherol and, therefore, a higher risk of increased systemic inflammation which, in turn, could lead to the disease progression. In this study that, to our knowledge, employs the largest database with reference methodology, we reported few cases of deficiency for both fat-soluble vitamins regardless the diagnostic of CD or UC. We conclude that supplementation only in cases where signs and symptoms of malabsorption are present could be an effective option.

**Bibliography**


**P14**

**THE IMPORTANCE OF BIOPATHOLOGICAL VALIDATION BY CLINICAL PATHOLOGIST – MULTIPLE MYELOMA CASE REPORT**

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**Introduction:** In January 2018, we received an EDTA blood sample from a 50-year old Caucasian male with a previous diagnosis of multiple myeloma (October 2015) that was under clinical surveillance after treatment with VCD protocol (cyclophosphamide, bortezomib and dexamethasone) and medullary auto-transplant (October 2016).

**Material and Method:** The sample was processed according to the laboratory standard procedures, using a Beckman Coulter LH 750 haematology analyser.

**Results:** Platelets were low (65G/l) and leukocytes elevated (21.3G/l), when compared to the previous results ranging between 100 and 140 G/l and 6,0 to 8,0 G/l, respectively. The report provided by the equipment did not include any platelet related alarms (“giant platelets” or “platelet aggregates”), so there was no suspect of blood clots. However, sample identification and integrity were confirmed, and a retest was performed. Both platelet and leukocyte counts confirmed the first values obtained.

The clinical pathologist selected this blood sample for smear, which elicited 2% plasma cells in peripheral blood. These finding led to flow cytometry immunophenotyping of the blood, with the identification of a plasma cell population with the same immunophenotypic characteristics reported in October 2015 (initial diagnosis). Posterior to these findings a bone marrow sample was collected and sent to the laboratory. Cell count, morphology and immunophenotyping revealed the presence of a similar plasma cell population observed in peripheral blood. During this clinical surveillance visit, the patient evidenced skin lesions in his back and shoulders that, after biopsy, were considered to be plasmacytomas.

**Conclusion:** This clinical case shows that automated validation should not be a routine practice; awareness of clinical pathologists can identify dubious cases. A critical mindset toward automated validation is essential, as it needs constant supervision and monitoring to evaluate if the assumptions that legitimized automated validation in the first place are solid through time.

**P15**

**HAEMOLYSIS IN CLINICAL CHEMISTRY - VISUAL INSPECTION VERSUS HAEMOLYSIS INDEX EVALUATION: OUR EXPERIENCE**

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**Introduction:** Pre-analytical errors account for 60-70% of laboratory errors1, being haemolysis the most frequent. Haemolysis interference can be attributed to different mechanisms such as spectral interference, chemical reactions between heme group and iron with reagents, release of intracellular contents, release of enzymes and dilution effect. In clinical chemistry laboratory, haemolysis is routinely evaluated by visual inspection. Thus, visual inspection method was directly compared to haemolysis index and the selection of collection repetitions for samples with increased haemolysis index (HI) was evaluated.

**Materials and Methods:** In a 1-month period (Oct 2017), all samples were visually inspected; samples with haemolysis were categorized as lightly haemolysed or haemolysed. HI measurements were performed in a Cobas® 6000 Analyzer Series equipment used in routine procedures. Collection repetitions were decided by staff according to visual inspection or suspected analytical interference. Statistical analysis was performed using t-test and Chi-Square Test with IBM® SPSS 23.0.
Results: Clinical chemistry sector received 6,118 samples whose analytical method required serum or plasma. 136 samples (2.2% of total) were considered to have some degree of haemolysis, being 86 classified as lightly haemolysed and 50 haemolysed. Collection repetition was performed in 33 (0.54% of total) samples: 3 in lightly haemolysed samples and 30 in haemolysed. Lightly haemolysed samples have an HI significantly lower than haemolysed ones (52.5 versus 140.2, p < 0.05). Samples that required a collection repetition have a significant increased HI (166.4 versus 58.5, p < 0.05).

Conclusions: According to these results, there is no difference between visual inspection and HI as both procedures enable the detection samples with higher haemolysis and that require collection repetition.

References

P16

VCS TECHNOLOGY POINTS OUT MORPHOLOGICAL CHANGES IN CHRONIC MYELOID LEUKAEMIA

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Introduction: Chronic myeloid leukaemia (CML) is characterized by a left shift with increased number of immature granulocytes and a maturation blockade. Translocation between long arms of chromosome 9 and 22 (BCR-ABL) is a cornerstone issue in CML as it is related to diagnosis, treatment and monitoring. Fluorescent in situ hybridization and reverse transcriptase-polymerase chain reaction are essential in detecting and quantifying BCR-ABL transcripts. VCS technology relates to morphological characteristics of leukocytes and has been proposed to distinguish patients with viral from bacterial infection and to support the diagnosis of sepsis. Herein, we aimed at establishing VCS differences between normal group and patients with CML, simultaneously exploring a possible relation of VCS with the quantification of BCR-ABL transcripts in peripheral blood.

Materials and Methods: In this study, 172 blood counts obtained from 12 patients between May 2014 and January 2018 were included. For each blood count, VCS related to neutrophils and monocytes; leukocyte, neutrophil and basophil counts were evaluated in a Beckman Coulter LH 750 haematology analyser. Whenever BCR-ABL quantification was ordered by the physician, this information was also included. Statistical analysis was performed through t-test and Pearson correlation.

Results: VCS parameters presented statistical significance (p < 0.05) regarding neutrophils and monocytes when comparing control group and CML group (volume, conductivity, and scatter, and respective standard deviation, SD), apart from scatter and SD of monocytes. This suggests an increased size and internal complexity of both populations in CML, but also an increased heterogeneity. The highest correlation between VCS and BCR-ABL quantification was detected in relation with neutrophils volume SD (r = 0.64) and conductivity SD (r = 0.63), suggesting that VCS is not appropriate to monitor treatment response.

Conclusions: Considering the characteristic left shift in CML, differences observed in VCS were expected. Nevertheless, a continuous investigation for new and more cost-effective methods for monitoring treatment response is needed.

References

P17

THE IMPORTANCE OF COLORECTAL CANCER SCREENING USING THE FECAL IMMUNOCHEMICAL TEST – EXPERIENCE A CENTRAL HOSPITAL

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Introduction: Colorectal cancer (CRC) is a leading cause of cancer deaths worldwide. Annual screening using fecal occult blood testing is recommended and used as an effective approach to reduce CRC incidence and mortality risk. The fecal immunochemical test (FIT) is a newer fecal occult blood test that uses a specific antibody for human hemoglobin. FIT is noninvasive and is more sensitive than traditional guaiac-based fecal occult blood test. Use of FIT is promoted by a several health primary care centers, to help increase screening rates for portuguese populations. In population-based screening programs, a FIT kit is delivery to patients that self-collect the sample and return it to the health centers for testing. This has enabled to screen large numbers of people without the need for a visit to the Hospital, promoting cost saving proximity healthcare.
Materials and Methods: The laboratory started receiving fecal samples in December 2017, sending by primary health centers. Samples were self-collected by the patients with indication to perform the CRC screening. Our laboratory, to date, has analyzed 128 samples using an automatic analyzer HM-JACKarc (A.Menarini, diagnostics) that uses antibodies to detect blood, more properly hemoglobin, in a small amount (2mg) of stool. The cut-off of the analyzer is 15 μg/g and gives results in just a few minutes.

Results: Patient’s ages were between 49 and 74 years. Samples above the cut-off were positive and from the total analyzed, 118 were negative and 10 positive. Only 8.4% of patients are indicated for medical additional studies.

Conclusions: This screening test is fast with high sensitivity and specificity and uses small amounts of sample that is simple to obtain. FIT will help to reduce patient travel to central hospitals, absences from work and reduces the availability of family members to accompany these patients. FIT represents a potentially viable, cost-effective option as a screening modality for CRC with a more favorable cost/detected case.

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P18
HEPARIN-INDUCED THROMBOCYTOPENIA – A CASE REPORT

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Introduction: Heparin-induced thrombocytopenia (HIT) type II is an important and frequent drug-induced, immune-mediated type of thrombocytopenia. It is mediated by heparin-platelet factor 4 (PF4) IgG antibodies which bind to heparin-PF4 complex, inducing thrombocytopenia and promoting a state of hypercoagulability that can result in thrombosis. The diagnosis is based on clinical assessment and laboratory testing. Clinical assessment is based on the use of the 4Ts score developed to improve and standardize clinical diagnosis and evaluates: (1) magnitude of thrombocytopenia; (2) timing of thrombocytopenia with respect to heparin exposure; (3) thrombosis or other sequelae of HIT; and (4) likelihood of other causes of thrombocytopenia for HIT. Laboratory testing is based on the determination of anti-PF4 antibodies. The authors present a case report of HIT upon a cardiac surgery.

Material and Methods: A 72-year-old man was admitted to the cardiothoracic surgery department for an elective surgery due to severe aortic insufficiency and ascending aortic aneurysm. The patient had history of type 2 diabetes, hypertension, obesity and dyslipidemia. At the admission, no significant alterations were detected on the blood tests. There was no previous history of HIT.

Results: The patient was submitted to a Bentall procedure without complications. Postoperatively, the patient was maintained on enoxaparin, 40mg, SC QD. Ten days after the surgery, the patient developed pericardial effusion, hemodynamic instability and atrial fibrillation that required cardioversion. Laboratory tests revealed a significant thrombocytopenia (a fall of 90% in platelet count) which raised the suspicion of HIT. The 4T score was 5, indicating an intermediate probability (~14% probability) of HIT. Anti-PF4 antibodies showed a positive result. Enoxaparin was interrupted and alternative anticoagulation was initiated. Platelet count progressively normalized and no thrombosis was evidenced.

Conclusions: Diagnosis of HIT in the postoperative setting of cardiac surgery is difficult since these patients usually present multiple causes for thrombocytopenia. The use of 4T score is important to establish the likelihood of HIT. Upon the diagnosis of HIT it is crucial to interrupt enoxaparin and to initiate an alternative anticoagulation.

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P19
EOSINOPHILIA IN CEREBROSPINAL FLUID: A LABORATORY FINDING - PRESENTATION OF A CLINICAL CASE

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Introduction: The presence of eosinophils in cerebrospinal fluid (CSF) is a rare laboratory finding and should always be considered abnormal. Eosinophilic meningitis is defined as the presence of >10 eosinophils/mm3 in the CSF and/or eosinophils accounting for >10% of CSF
leukocytes. Although parasitic helminthic infections are the most frequent cause of this type of meningitis, other etiologies must be considered, namely other infections (fungal, bacterial and viral) and non infectious causes, such as neoplastic diseases, medications and prosthesis reactions.

**Materials and Methods/Results:** The authors present the case of a 46 year old woman recently submitted to meningioma surgery and ventriculoperitoneal shunt implantation, presenting with fever and uncontrollable vomiting. She was admitted to the Neurotrauma Service with suspected shunt dysfunction versus CSF fistula.

Urgent cytochemical CSF analysis was request and revealed: high CSF protein (301 mg/dL); normal CSF Glucose (59 mg/dL); CSF leukocytes: 261/mm3 with 55% eosinophils.

CSF bacteriological examination was negative.

She was re-operated with abdominal tip replacement - with functioning shunt - in the peritoneum.

In this case, the laboratory tests results were fundamental to the clinical conduct followed.

**Conclusions:** The diagnosis of eosinophilic meningitis rests on the integration of the patient's clinical manifestations and epidemiologic data with the microscopic identification of eosinophils in CSF.

Routinely, CSF cellular examination and cell counts are usually done with fresh, unstained fluid samples, and eosinophils are difficult to identify in these preparations.

Laboratory staff should be aware of the importance of correct differentiation of leukocytes in CSF on stained lamina (Giemsa or Wright stain) after cytocentrifugation that permits a correct differential diagnosis with the other much more common etiologies of meningitis.

**References:**


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**EXTRAMEDULLARY PLASMACYTOMA OF THE PANCREAS – A CASE REPORT**

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**Introduction:** Extramedullary plasmacytoma represents less than 5% of all plasma cell neoplasms and, within these, only 10% occur in gastrointestinal tract.

**Materials and Methods:** 44 years old male patient, referred to our hospital with right shoulder pain, left thigh pain, nocturnal sudoresis and significant weight loss, with 2 months of evolution; imagiology evaluation showed multiple cervical and supraclavicular adenopathies as well as innumerous osteolytic lesions affecting the whole skeleton.

**Results:** The analytical study performed in the Clinical Pathology Department evidenced the presence of erythrocyte rouleaux in peripheral blood smears; hypercalcemia, hyperproteinemia, hyperuricemia and high lactate dehydrogenase; elevated IgG (2690mg/dl), free Kappa/Lambda ratio (186,16) and β-2-microglobulin (5,96 mg/dl). Serum protein electrophoresis and immunofixation revealed a predominant IgG kappa monoclonal band as well as a discrete free light kappa chain monoclonal band. 24-hour urine presented with proteinuria of 2601 mg and presence of monoclonal free light kappa chain. Bone marrow cell differential count showed plasmocyte infiltration (10%), confirmed by flow cytometry immunophenotyping study, with 99,5% of these showing abnormal phenotype. FISH studies revealed gain of 1q21 region in 80% of plasmocyte nuclei. Integrating all the data available (clinical, imagiology and laboratory studies) the patient was diagnosed with stage III multiple myeloma IgG kappa. CyBorD protocol was initiated (Ciclofosfamide, Bortezomib and Dexamethasone) as well as antalgic radiotherapy. A PET scan and MRI were also requested, revealing pancreatic nodular lesions (suspicious of high metabolic rate neoplasia or secondary lesion). Immunophenotyping of ultrasound guided endoscopic biopsy of pancreatic nodules revealed multiple myeloma infiltration (87% of plasmocytes).

**Conclusions:** Pancreatic extramedullary plasmacytoma is an extremely rare manifestation associated to multiple myeloma that should be considered in differential diagnosis of pancreatic masses. Immunophenotyping is an essential tool, along with other classic investigation methodology, for this pathology definitive diagnosis, demonstrating extramedullar monoclonal plasmocytes.

**Bibliographic references:**

P21


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Introduction: Meningitis is a medical emergency. Immediate diagnostic steps must be taken to establish the specific cause so appropriate antimicrobial therapy can be initiated. The morbidity and mortality caused by this entity can be reduced with an early accurate diagnosis. The new fast molecular biology tests have increased the diagnosis specificity, identifying the most common agents.

Case report: A 19-month-old male was observed by the family paediatrician for prostration, fever and anorexia. Amoxicillin/clavulanate was initiated for an acute tonsillitis. On the next day, the child was brought to the emergency room due to health state aggravation and frequent vomiting. He was prostrated but conscious and collaborative, dehydrated and with fever (38 °C). The remainder physical examination, including neurological evaluation, was unremarkable. Analytic study was conducted: 13g/dl haemoglobin, 9800/µL leucocytes, 24600 platelets; glucose 140mg/dL, normal ions and kidney function; C-Reactive Protein 139 mg/L. Type B urine was unremarkable as well as thorax X-ray. Supportive therapy was initiated. Without any improvement in the early minutes, the child was admitted in the infirmary and a lumbar puncture was done. The cerebrospinal fluid (CSF) analysis revealed 5800 leucocytes/µL with 95% polymorphonuclear cells; glucose under the limit of detection and 36760 mg/dL protein. The pathologist requested a gram stained direct exam and observed gram negative diplococci. CSF was tested with Meningitis/Encephalitis panel in the multiplex PCR system FilmArray® (BioFire Diagnostics) resulting in the detection of Neisseria meningitidis. Ceftriaxone was initiated as well as prophylactic antibiotics for the contacts. N. meningitidis was also found in the CSF culture 24h later and antibiogram was performed. Type B N. meningitidis was also identified. The patient had a good clinical outcome, without complications, and was discharged after 7 days.

Conclusion: This case emphasizes that fast PCR tests allow an early diagnosis, making possible the start of the correct treatment, diminishing mortality and sequelae for patient. Prophylactic treatment for health care professionals and other contacts as well as the notification of these type of disease can be done earlier, avoiding the possibility of an outbreak.

P22

EVALUATION OF PROLONGED aPTT IN CHILDREN

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Introduction: Activated partial thromboplastin time (aPTT) is used to evaluate the intrinsic pathway of coagulation. In pediatric patients, the incidental detection of a prolonged aPTT is a concern, since the test is commonly requested without a history of bleeding and frequently as preoperative evaluation. A prolonged aPTT leads to extensive and repeated laboratory tests and frequently to a referral to the pediatric hematology department. It was our aim to retrospectively review the results of the pediatric population in order to better understand the causes of prolonged aPTT.

Material and methods: The results were collected anonymously from our hospital laboratory middleware database during a 4 years period. Age, sex, diagnosis and results from hemostasis evaluation were collected. The study was limited to children aged between 6 months and 17 years old. Data were analyzed with SPSS software.

Results: During 4 years were performed 2787 determinations of aPTT that correspond to 788 patients. From those, 321 patients (40.7%) presented aPTT prolongation. 129 (40.2%) out of 321 patients were at the moment evaluated for lupus anticoagulant (LA). In 95 (73.6%) out of 129 patients the tests were requested as preoperative evaluation. In 25 (26.3%) of the 95 patients, LA was also positive. These results were confirmed in a second sample, collected a few hours later, to exclude pre-analytical errors: 10 patients maintain LA positivity that was associated to an inflammatory response in the context of the illness and did not continue the coagulation study; 3 patients maintained the prolonged aPTT and confirmed the presence of LA in subsequent samples; 2 patients maintain a prolonged aPTT and became LA negative which lead to the identification of factors deficiency; and 10 patients normalized the aPTT and became LA negative.

Conclusions: A significant percentage (40.7%) of children presented a prolonged aPTT. In children under preoperative study the prolonged aPTT was associated to pre-analytical errors, inflammatory response in the context of the illness, coagulation factors deficiency and to the presence of LA. The results emphasizes the role of pre-analytical variables in the coagulation tests as well as the need to confirm the results before the referral to the pediatric hematology department.

P23

ESTIMATING GLOMERULAR FILTRATION RATE FROM SERUM AND URINARY CREATININE AND SERUM CYSTATIN-C: A COMPARATIVE STUDY

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Introduction: The laboratory evaluation of Renal Function is essential in diagnosis and follow up of chronic renal disease (CKD). Glomerular Filtration Rate (GFR) is routinely estimated by equations that use serum (s) creatinine (Cr), which are imprecise, potentially leading to overdiagnosis. Cystatin-C (Cys) can be an alternative or complementary marker in this evaluation. The aim of this study was to find out if equations that use sCys solely or combined with sCr tend to be more precise than those that use Cr alone, in our sample.

Material and methods: A cross-sectional study was performed on a sample of 164 ambulatory adult patients. sCys was analyzed by nephelometry and Cr (serum and urinary) by spectrophotometry-Jaffe method, both in Dimension Vista ®1500 System, Siemens equipment.

We calculated and compared the performance of five formulas: Chronic Kidney Disease – Epidemiology Collaboration (CKD-EPI) Cys, CKD-EPI Cr, CKD-EPI CrCys, Modification of Diet in Renal Disease (MDRD) and Cr Clearance in 24h urine equation (ClCrU24hArea), using the latter as a reference.

Results: Our sample was heterogeneous with 49.39% of our patients presenting GFR ≥90 mL/min/1.73 m², 23.78% GFR of 60 to 89 mL/min/1.73 m², 20.73% GFR of 30 to 59 mL/min/1.73 m² and 6.10 % GFR < 30 mL/min/1.73 m². The mean ± standard deviation (SD) of ClCrU24hArea was 92.02 (±45.75) mL/min/1.73 m².

When comparing this results with those obtained by the use of other estimating formulas, we verified discordant results in 41.46% (CKD-EPI Cr), 31.10 % (CKD-EPI Cys), 35.37% (CKD-EPI CrCys) and 51.83% (MDRD) of our sample (p < 0.001 for all the comparisons).

Conclusion: Although our study presents some limitations such as not using the clearance of an exogenous marker as a standard, we verified that the equation which combines Cr and Cys provides the most concordant results and MDRD the most discordant, with the formula we considered as reference.

On other hand, and despite recent advances have facilitated the use of Cys as a marker of kidney function, we cannot fail to mention that the costs associated with this marker are high, therefore we should limitate its use to cases where there is a cost-benefit.

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P24

PREVALENCE OF EXTENDED-SPECTRUM BETA-LACTAMASES PRODUCING ENTEROBACTERIA IN A COMMUNITY LABORATORY

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Introduction: Study the prevalence and antibiotic susceptibility profiles of some Enterobacteriaceae (Escherichia coli and Klebsiella pneumoniae) isolated from urinary patients over a period of 6 years in a Community Laboratory.

Materials and Methods: In the period from January 2012 to December 2017, 7654 strains of E. coli and K. pneumoniae, were recovered from urinary samples, obtained from outpatients of the Laboratory J. Leitão Santos in Alverca.

Identification and sensitivity to antibiotics of all strains were carried out by the Vitek 2 system (bioMérieux, Marcy L’Étoile, France) according to the Eucast recommendations.
The strains were identified as extended spectrum beta-lactamases (ESBL) producers using the AST cards (for aerobic gram negative bacilli) and the Advanced Expert System (Vitek 2 AES).

**Results:** During the observation period, 6284 strains of *E. coli* and 1170 of *K. pneumoniae*, in the total of 7454 clinically strains were recovered from urinary patients. A total of 696 ESBL positive strains (9.3%) were detected, out of which: *E. coli* – 480 (7.7%) and *K. pneumoniae* – 216 (18.5%). Among all ESBL positive strains, Nitrofurantoin resistance occurred in 1.7% of *E.coli* and in 52% of *K.pneumoniae* and Fosfomycin resistance occurred in 11% of *E.coli* and 36% of *K.pneumoniae*. Co-resistance of isolated ESBL positive strains to Aminoglycosides and Fluoroquinolones were frequent: 79% of *E.coli* and 83% of *K.pneumoniae* were resistant to Fluoroquinolones, and 33% of *E.coli* and 48% of *K.pneumoniae* were resistant to Gentamycin.

**Conclusion:** The high prevalence of ESBL producing strains of Enterobacteria suggests the need of ESBL screening on regular basis. High degree of resistance was exhibited to Fluoroquinolones and Gentamycin. Nitrofurantoin and Fosfomycin are a good therapeutical option for *E. coli* ESBL producers.

**References:**

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**P25**

**HELICOBACTER PYLORI PREVALENCE STUDY IN BREATH TEST SAMPLES**

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**Introduction:** *Helicobacter pylori* is a gram-negative microaerophile bacterium which can be found on the gastric mucosa and which is presently considered to be a main cause of chronic gastritis, peptic ulcer and gastric carcinoma. However, it is estimated that as much as 80% of the infected individuals remain asymptomatic, which leads to believe that this organism can play a yet undefined role in gastric biology.

**Materials and methods:** The respiratory test is a test in which a small quantity of 13C urea is ingested and a breath sample is collected about half an hour later. In infected patients the microorganism’s urease degrades the 13C urea releasing 13CO2 and ammonia in the blood stream which are then eliminated through the lungs with the expired air. Since urease is absent in the normal gastric bacterial flora, the test is reasonably specific for *Helicobacter pylori*.

The breath test results were collected from the laboratory’s computer covering a seven-year period (Jan’2010 to Feb’2018) and prevalence was calculated for the positive samples according to sex and age.

**Results:** 345 results were included and segregated into age groups (<20 years old patients, 32 samples; 21 to 30 years old, 102 samples; 41 to 60 years old, 160 samples and >60 years old, 51 samples), with a sex distribution of 66.4% female and 33.6% male. The positivity rate of the studied population is 32%. However, this value cannot be taken as representative of the whole population because only samples from referenced patients were studied.

**Discussion:** No % differences between both sexes were observed, as expected. Also, no significant differences were detected between age groups, a finding also in consonance with the literature. The fact that most samples come from the group of 41 to 60 years old patients could point out the major group for the occurrence of clinical symptoms which could be associated with the *Helicobacter pylori* infection. The main conclusion is that the infection by *Helicobacter pylori* is transversal to the whole population regardless of sex and age.

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**P26**

**INTESTINAL PARASITES: REALITY OF MADEIRA ISLAND 2010-2016**

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**Introduction:** The fecal parasitic examination studies are still a relevant diagnostic tool, giving important information in the diagnosis and screening of populations. The purpose of this study is to know the reality of intestinal parasitic infection in Madeira Island, regarding fecal...
specimens analysed in the Clinical Pathology Department of SESARAM, EPE (Serviço de Saúde da Região Autónoma da Madeira) using a modified Ritchie technique, between 2010 – 2016.

**Material and methods:** Data about fecal parasitic examination requested by Hospital Clinical Services and Community Health Centers of SESARAM, EPE, between 2010 – 2016 was collected using *Werfen Modulab*® v2.3.08 software. We collected the following data: age, sex, clinical department, number of samples and parasitic examination result. *Microsoft Excel 2013*® was used for statistical analysis.

**Results:** Out of 14738 requests 268 had positive pathogenic parasite identification (1.8%). The most frequently found parasites were: *Giardia lamblia* (55.6%), *Strongyloides stercoralis* (13.1%), *Taenia spp.* (11.2%) and *Ascaris lumbricoides* (11.2%). In 31 requests more than one parasite was found. Regarding samples from Community Health Centers, Funchal was the district with most requests (n = 3215). Santana (2.7%), Ponta do Sol (2.4%) and Câmara de Lobos (2.2%) had the highest rate of positivity. The average age for positive results was 41.3 years old (total average 40.78 years).

**Conclusions:** Since this is the first study about of intestinal parasitic infection in Madeira Island it is not possible to make comparisons regarding prevalence or temporal differences. Despite this, we found a low rate of intestinal parasitism which is in agreement with the Portuguese reality. The highest rate in Madeira Island was found in rural areas.


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**P27**

**THE IMPORTANCE OF PHARMACOGENETICS IN CLINICAL PRATICE - CASE REPORT**

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**Introduction:** Pharmacogenetics studies the genetic factors that influence drug response and toxicity. The genes encoding the enzymes cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKOR) are the most correlated genetic factors with warfarin dose requirements. Patients receiving warfarin who have one or more genetic variations in CYP2C9 and VKORC1 are at increased risk of adverse drug effects and require significant dose reductions to achieve a therapeutic international normalized ratio (INR).

**Case Report:** The authors report a clinical case of warfarin hypersensitivity in a 40-year-old male patient with thromboembolic risk. He was admitted to our hospital with hemoptoic sputum and hematuria. He had a recent past medical history of atrial fibrillation treated with warfarin 5 mg/day since one week before his hospital admission. Laboratory investigations revealed anemia, elevated prothrombin time (PT) (119s), INR (11.17) and activated partial thromboplastin time (APTT) (61.3s). Chest radiography and abdominal ultrasound were normal. Vitamin K and plasma were infused along with discontinuation of warfarin. Even with this treatment he was still bleeding and with elevated PT (65.6s), INR (5.83) and APTT (39.5s). Genetic analysis later revealed that he was homozygotic to 1075A>C (genotype CYP2C9*3/*3) and heterozygotic to -1639G>C (gene VKORC1). After stabilized INR and stop bleeding, warfarin dosing was subsequently restarted and stabilized at 1.25 mg daily with therapeutic INR.

**Conclusion:** This case highlights the importance of pharmacogenetic testing in clinical practice to maximize the efficacy, minimize the risk of adverse drug effects and prevent fatal outcomes.

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**P28**

**AUTOIMMUNE POLYGLANDULAR SYNDROME TYPE 3 - CLINICAL CASE**

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**Introduction:** Autoimmune polyglandular syndrome (APS) consists in an association of two or more autoimmune diseases (AID) and is classified into four types. Type 3 APS is characterized by autoimmune thyroid disease associated with other autoimmune diseases, excluding the involvement of the adrenal gland. The authors present a clinical case of APS type 3 (autoimmune thyroiditis (AIT), autoimmune hepatitis (AIH) and primary biliary cholangitis (PBC)), in which overlapping pathologies present a diagnostic and therapeutic challenge.

**Clinical Case:** A 47-year-old female patient was admitted in the Emergency Department with abdominal pain and lower limb oedema. She had a history of AIT and hypothyroidism treated with levothyroxine. No history of travel or consumption of hepatotoxic products. She had sub-icteric sclerotics and lower limb oedema. Laboratory investigations revealed elevated hepatic enzymes, hepatic insufficiency, pancytopenia, polyclonal hypergammaglobulinemia and elevated thyroid stimulating hormone, with normal free T3 and T4 levels. Anti-nuclear antibodies were positive with dense fine granular pattern, as well as anti-mitochondrial, anti-pyruvate dehydrogenase IgG-M2, anti-Gp210, anti-thyroglobulin and anti-peroxidase antibodies. Anti-LKM (liver kidney microsomes), anti-smooth muscle and anti-actin antibodies were negative. Hepatitis B and C screening was negative. Abdominal ultrasound revealed moderate peritoneal fluid with signs of chronic hepatic
disease, without dilatation of the intra and extrahepatic bile ducts. Hepatic biopsy was compatible with AIH. The patient was treated with prednisolone, ursodeoxycholic acid, azathioprine and levothyroxine.

**Conclusion:** The association between AIH, PBC and other AID is rare. The laboratory study allowed the diagnosis of PBC and AIT, however the hepatic histology was compatible with AIH. This clinical case highlights the rarity and difficulties in diagnosis, being a timely adequate treatment essential to survival improvement.

**P29**

**A SIMULATION METHOD FOR INVESTIGATING THE IMPACT OF ANALYTICAL PERFORMANCE ON THE PROBABILITY OF PATIENT OUTCOMES? ABOUT A CASE ON A HOSPITAL CENTER’S LABORATORY**

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**Introduction:** The 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine defined the Consensus on analytical performance specifications for clinical laboratories (CAP). The CAP’s model 1b, “Based on the effect of analytical performance on clinical outcomes”, states that it can be achieved by simulation of analytical performance on the patients results. At Centro Hospitalar de Leiria’s clinical pathology service, patients distribution data’s charts on Modulab Gold (PDC) are evaluated daily to ascertain the analytical methods stability along the internal quality control with commercial samples. This work shows how an internal error leaded us to propose implementing different analytical requirements directly on quality control charts and evaluating it’s impact on PDC, as a method for CAP’s model 1b.

**Material and Methods:** Biological variation is widely studied has a reference for analytical performance specification to clinical laboratories. After an error with the potassium measurement method in our laboratory, we observed the potassium PDC in two situations of allowable maximum uncertainty measurement on our Internal Quality Control charts with a maximum CV% of 5% and 3% for commercial samples.

**Results:** We observed that with a systematic error of 5%, the PDC showed a shift on the patients distribution for potassium. We also observed a normalization of the potassium’s patient distribution, when we tightened the maximum allowable error to 3%.

**Conclusion:** When we implement a quality control program with a maximum allowable uncertainty of 3% for potassium, it respects the “thumb rule” of setting the analytical noise under the intraindividual biological variation (VBi) wich is 4,6% for potassium, and we confirmed to be safe for the patients results. Whereas a 5% error, despite being under interindividual biological variation for potassium wich is 5,6%, we confirmed it affects the patients. This case suggests that any laboratory can use it’s own PDC along the commercial control sample, inducing different bias to define the maximum analytical error that does not affect the patients results, as a method to define a CAP’s model 1b analytical performance specification.

**References**


**P30**

**EVALUATION OF VITEK MS SYSTEM FOR IDENTIFICATION OF NOCARDIA**

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**Introduction:** MALDI-TOF mass spectrometry (MTMS) for identification of nocardia remains challenging. To improve identification, previous studies used modified sample preparation methods and the databases have been improved. We show the performance of one of these methods for the identification of nocardia by VITEK® MS (VMS) (BioMérieux, França).

**Material and Methods:** Sixty-six isolates (Nocardia nova, n = 56; Nocardia farcinica, n = 5; Nocardia shimonofusensis, n = 1; Nocardia rhambnosphila, n = 1; Nocardia cyriacigeorgica, n = 1; Nocardia brevicatena, n = 1; Nocardia brasiliensis, n = 1) identified by 16S rRNA gene sequencing were tested by VMS after the following extraction method: colony were suspended in 300 μL of 0.5% Tween-20; boiled for 30 min; centrifuged at 13000 rpm for 2 min; pellet was vortexed at 6000 rpm for 60 sec with 500 μL of distilled water and zirconium oxide beads
in MagNA Lyser (Roche, Germany); centrifuged at 13000 rpm for 2 min; pellet was resuspended in 300 μL of distilled water and 900 μL of ethanol; two series of centrifugations at 13000 rpm for 2 min were performed to remove the supernatant completely; 50 μL of 70% formic acid was added and incubated for 15 min; 50 μL of 100% acetonitrile was added; centrifuged at 13000 rpm for 2 min and 1 μL of supernatant was tested. The spectra were analyzed using VITEK MS (IVD) Base de dados v3.

**Results:** VMS correctly identified 66.7% (44 of 66) of the isolates at species level. Additionally, a specificity of 100% was found.

**Conclusions:** Considering 16S rRNA sequencing as a reference method, VMS showed to be a promising tool for routine identification of Nocardia species, mainly in a context where other methodologies such as gene sequencing may not be available. As other studies proved, its performance can still be improved by the use of commercial extraction kits specially developed for nocardia and mycobacteria. In future studies we will analyze these commercial alternatives.

**P31**

**RELEVANCE OF N-TERMINAL PRO-B TYPE NATRIURETIC PEPTIDE MEASUREMENT IN SUSPECTED ACUTE HEART FAILURE AT THE EMERGENCY DEPARTMENT**

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**Background / Aim:** The approach to the patient with suspected acute heart failure (AHF) should comprise both clinical history and diagnostic tests (electrocardiogram, chest radiograph and blood tests). B-type brain natriuretic peptide (BNP) is mainly secreted from the ventricles and released due to volume expansion or pressure overload. Cleavage of the pro-hormone (proBNP) generates biologically active BNP and inert N-terminal pro-BNP (NT-proBNP). The aim of this study was to assess the relevance of NT-proBNP measurement in suspected AHF at the emergency department (ED).

**Materials / Methods:** Observational, cross-sectional, retrospective and descriptive study. Data collection included all suspected AHF episodes from the ED of our hospital between April and December 2017. NT-proBNP measurement was performed on serum samples by an electrochemiluminescence immunoassay on the MODULAR E170 (Roche) analyzer and, according to the 2016 European Society of Cardiology guidelines, the cut-off value was 300 pg/mL. Data analysis was carried out through Microsoft Excel® 2010 (Microsoft Corporation).

**Results:** A total of 1452 emergency episodes from subjects with a mean age of 76 years were included; 43.0% (n = 624) were male and 57.0% (n = 828) female. The clinical manifestations that triggered NT-proBNP measurement were acute dyspnoea in 79.5% (n = 1155), crackles on pulmonary auscultation in 72.8% (n = 1057), orthopnoea in 69.6% (n = 1011), lower limb oedema in 54.1% (n = 785), paroxysmal nocturnal dyspnoea in 33.7% (n = 489), increased jugular turgor in 24.9% (n = 361), S3 on cardiac auscultation in 11.4% (n = 165) and other causes (such as pleural or peritoneal effusion) in 3.9% (n = 56). NT-proBNP ≥ 300 pg/mL supported the diagnosis of AHF in 80.5% (n = 1169) while NT-proBNP < 300 pg/mL helped to exclude it in 19.5% (n = 283).

**Discussion / Conclusion:** When requested on the basis of a suggestive clinical history, NT-proBNP measurement appears to be an excellent biomarker of AHF and thus to enhance the diagnostic accuracy and reduce healthcare-associated costs. Nevertheless, NT-proBNP should not be used alone to diagnose AHF, as low levels have been reported in very AHF (first hour) and obesity whilst high levels may be associated with a large variety of cardiac (such as atrial fibrillation) and non-cardiac disorders (such as renal failure).

**P32**

**THE IMPORTANCE OF MOLECULAR AND CONVENTIONAL CYTOGENETIC IN ACUTE PROMYELOCYTIC LEUKEMIA PROGNOSTIC - CLINICAL CASE**

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**Introduction:** Translocation (15;17) leading to formation of fusion gene PML/RARα is the diagnostic hallmark of acute promyelocytic leukemia (APL). Interphase fluorescence in situ hybridization (FISH) is one of the diagnostic tools employed for the detection of this fusion gene. Using a dual color dual fusion (D-FISH) PML/RARα translocation DNA probe, it is possible to characterize the FISH pattern of APL. However, the diagnosis of PML/RARα in APL should not be solely relied on D-FISH because in case of additional changes or complex karyotypes, atypical patterns are observed which can lead to uncertainties in interpretation.
Clinical case: A 57-year-old male, with a personal history of type 2 diabetes mellitus, kidney failure, left sided hemiparesis, dysarthria and epilepsy after ischemic stroke, was transferred from another hospital with APL suspicion. On admission, he was dysarthric and left hemiparetic with arms bruises. Laboratory investigation revealed pancytopenia, elevated prothrombin time, d-dimers, creatinine, liver enzymes and uric acid. There were 55% promyelocytes in the peripheral blood smear. The immunophenotypical study revealed 56% myeloid blasts with neutrophil maturation, most of them in the promyelocyte stage, not expressing CD15, suggestive of APL with t(15;17). Molecular biology was positive for bcrl/bcr2 transcript of the PML-RARα fusion gene (quantification in progress). D-FISH with the LSI t(15;17)PML/RARα probe performed in blasts HLA DR and DR+ detected t(15;17)(q24;q21) PML-RARα, predominantly with atypical patterns, in 90% of the nuclei. The conventional cytogenetic study revealed a complex karyotype (69,XY:t(15;17)(q24;q21);del(11)(q14;q23);del(20)(q11;q12);+8; +21; +mar[10]). Currently, the patient is still hospitalized and dependent on blood transfusions and all-trans retinoic acid (ATRA).

Conclusion: A correct diagnosis of APL with PML/RARα fusion is important since a high remission rate and improved survival can be achieved using ATRA or arsenic trioxide containing regimens. However, clinical significance of additional chromosomal abnormalities remains controversial, since it is not established prognostic significance of additional conventional and molecular cytogenetic abnormalities.

P33

ANTI-Xa IN CLINICAL PRACTICE - RETROSPECTIVE STUDY

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Introduction: Heparin is the most frequently used antithrombotic drug. The anti-Xa is a chromogenic assay based on a synthetic chromogenic substrate and on factor Xa inactivation. First, heparin is analyzed as a complex with antithrombin present in the sample. Second, the factor Xa is neutralized by heparin-antithrombin complex and residual factor Xa is quantified with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the heparin level in the sample. Although several studies have evaluated low-molecular-weight heparin (LMWH) dose adjustment methods based on anti-Xa levels, no definitive conclusion as yet exists.

Material and Methods: Retrospective analysis of the clinical and laboratory data of all patients with anti-Xa test performed between 2008 and 2017, irrespective of the diagnosis and measures taken in cases of values out of reference values (0.3-1 IU/ml).

Results: It were performed 1108 anti-Xa tests, 628 (57%) were out of the reference values, 32 were higher than 1 IU/ml and 559 were lower than 0.3 IU/ml. It was included 470 patients (290 males/180 females). The average age was 58 years (1 week to 96 years old). The majority anti-Xa tests were performed on hospitalized patients (1037), mostly from hepatic transplantation unit (442) and intensive care (359) doing LMWH in therapeutic doses. Only in 30% and 53% of the cases of lower and higher levels of anti-Xa respectively, the test was repeated to confirm the correct dose of LMWH. Renal impairment was not a significant impetus for testing, since the majority of patients had normal glomerular filtration rate.

Conclusions: Based on our study it is apparent that anti-Xa concentrations are often drawn inappropriately in clinical practice, especially out of transplantation or intensive care units. It is important to properly educate health care prescribers on correctly ordering anti-Xa levels since this laboratory test is expensive, and it is important to be able to utilize the result to guide dosage adjustment. This illustrates uncertainty of interpretation and clinical impact of routine anti-Xa testing, as management was affected in only 42% of the patients with levels out of reference values. It is not yet clear in which clinical context providers should send anti-Xa levels.

P34

ASPURIUS THROMBOCYTOPENIA: WHEN ASTRONOMY MEETS HEMATOLOGY

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Introduction: Platelet satellitism (PS) is the in vitro adherence of platelets to leucocytes. It is observed on blood smears prepared from blood collected in EDTA and usually it is absent with citrate or heparin. Two hypothesis were posed to explain the mechanism: Two main hypothesis have been posed to explain the mechanism underlying this phenomenon: one states that there is an EDTA-dependent binding of serum IgG antibodies to both the platelet glycoprotein IIb/IIIa complex and neutrophil Fcg-receptors and the second poses a non-immunologic adherence mediated by thrombospondin. We aimed to present a case-report of PS in a patient diagnosed with chronic secondary immune thrombocytopenia (ITP).

Material and Methods: A 59-years-old male patient was diagnosed in 2010 with ITP. The patient was previously diagnosed in 2004 with B-cell non-Hodgkin lymphoma and was in complete remission after chemotherapy and splenectomy since 2008.
Results: The complete blood count showed no alterations other than thrombocytopenia (56 G/L). Due to clinical history a blood smear was analyzed. The blood smear obtained from EDTA anticoagulated blood revealed the presence of 4-9 platelets attached circumferentially to most neutrophils. No platelet aggregation around other cells or isolated was seen. The estimated counting of platelets in the blood smear was 110 G/L. The observations were consistent through the time. Several laboratory tests were performed to study the PS. Antiplatelets antibodies and cryoglobulins screening was negative. Sedimentation rate was persistently below 5mm/hr. Antinuclear antibody investigation and direct and indirect Coombs tests were negative. Serum protein electrophoresis and immunofixation show no alterations. Serology for herpes simplex, Epstein-Barr, hepatitis and human immunodeficiency virus, cytomegalovirus, toxoplasma, and Treponema pallidum was negative.

Conclusions: PS constitutes a cause of pseudothrombocytopenia and if not identified can lead to inappropriate treatment. Contrary to the literature, PS was maintained over the years. The identification of PS was only possible due to blood smear examination since no flag other than the associated thrombocytopenia was displayed by the hematology analyzer.

References

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SHOULD WE CARE ABOUT PLATELETS IN TYPE 2 DIABETES PATIENTS?

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Introduction: Type 2 diabetes mellitus (T2DM) is a chronic, slowly progressing and systemic disease with increasing prevalence. Environmental and genetic factors contribute to the insulin resistance (IR) and impaired glucose homeostasis that characterize this disease. The mechanisms of IR seem to alter the neurovascular unit and activate adipocytes leading to the development of a chronic low-grade inflammatory state. More recently, studies showed that T2DM is also associated to platelets dysfunction resulting in haemostasis changes and inflammation. Utilizing glycated haemoglobin (HbA1c) as a marker of long-term glycemic control we aimed to assess the inflammation presence in T2DM patients, by retrospectively revising several parameters namely, leukocytes (Lk) and neutrophils (Nt) count, C-reactive protein (CRP), plateletcrit (Plt) and mean platelet volume (MPV) as an indicator of platelet activity.

Material and Methods: One-year results were collected, anonymously, from our laboratory information system database. Along with the laboratory data, age and diagnosis were also collected. Patients under 44 years of age were excluded, as the ones with CRP above 1 mg/dL. The patients were then separated in two groups: group 1 with HbA1c ≤ 6.5%; group 2 with HbA1c > 6.5%. Data was analysed with SPSS software.

Results: In group 1 (8213 patients) the average HbA1c was 5.8% ± 0.4 while in group 2 (5597 patients) it was 7.9% ± 1.2. The Lk count [6.8 ± 2.1 vs 7.8 ± 5.3 (P < 0.001)], the Nt count [58.7 ± 9.7 vs 59.3 ± 9.8 (P = 0.003)], the MPV [8.9 ± 1.1 vs 9.0 ± 1.3 (P < 0.001)], the Plt [0.179 ± 0.0510 vs 0.187 ± 0.0537 (P < 0.001)] and the CRP [0.29 ± 0.53 vs 0.36 ± 0.72 (P < 0.001)] were significantly higher in samples with HbA1c > 6.5%. The platelet count showed no statistic difference between groups. The analysis of the correlation between HbA1c and the parameters showed a statistically significant positive correlation for Lk count, MPV, Plt and CRP.

Conclusion: These results confirm that in T2DM there is an increase of several inflammatory markers and that there are changes that involve the platelets, namely the VPM, which may represent a surrogate marker of platelet activity. Further studies are needed to understand the eventual role of platelets in the inflammatory microenvironment that characterize T2DM.

References

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KIDNEY STONES - A RETROSPECTIVE ANALYSIS OF 10 YEARS

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Introduction: The incidence of nephrolithiasis is increasing, mainly in developed countries, with estimates of about 10% of the population having ≥1 episodes, associated with high recurrence rates. The composition of urinary calculi is variable and different constituents may be present, with calcium oxalate (CaOx) being the most frequently found, followed by uric acid (UA) and struvite. Lithogenesis is influenced, among others, by age, sex, and diet.
Methods and Materials: Retrospective study, based on the analysis of lab data from all uroliths studied in our biochemistry department, from January 2008 to December 2017. Stone analysis was made through semi-quantitative colorimetric detection using the LTA Kidney Stone Analysis Kit.

Results: 909 stone samples were analyzed, 58.9% corresponding to men (n = 535) and 41.1% to women (n = 374) with a mean age of 53.6 years. The most frequent type of urolith was CaOx with a frequency of 44.9% (n = 408), followed by 25.7% of UA (n = 234), 18% CaOx with phosphate (n = 164), 5.9% calcium phosphate (n = 54), 2.1% cystine (n = 19), 1.8% CaOx and UA (n = 16) and 1.5% struvite (n = 14). According to gender the most frequent compounds found in stones were, in males: 47.5% CaOx (n = 254), 30.8% UA (n = 165) and 13.1% CaOx with phosphate; and in females 41.8% samples with CaOx (n = 154), 25.1% CaOx with phosphate (n = 94) and 18.5% UA (n = 69). Analysis according to age group showed 235 cases (25.9%) between 50-60 years old, 182 (20%) in 60-70 years, 166 (18.3%) in 50-50 years, 140 (15.4%) between 30-40 years, 130 (14.3%) in people >70 years old, 45 (4.9%) in ages from 20-30 and 11 (1.21%) from 0-20 years old. In the 0-20 years age group the most frequent type of urolith was CaOx with phosphate (65.5%) and in the >70 age group majority of stones were UA (60.8%). In the remaining age groups CaOx uroliths were the most frequent (46.6%).

Conclusion: Analysis of our sample reflected a higher frequency of uroliths in men with the most frequently encountered type being CaOx-containing stones and struvite the least frequent. The peak age group for incidence of stones was 50-60 years in both genders. To our knowledge the results of our study are in line with current literature. Stone compound analysis allows a better understanding of the mechanisms involved in lithogenesis, which is important for treatment and prevention of recurrences.


P37

LEGIONELLA PNEUMOPHILA ISOLATION ON CHOCOLATE AGAR

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Introduction: Legionella pneumophila is an aerobic, Gram negative and intracellular facultative agent, responsible for a severe form of pneumonia. (1) Although cultural methods remain the gold standard for detection, some microbiology laboratories don’t have access to enriched media used for primary isolation of Legionella species. (2) Here we present a case of an intensive care unit patient with respiratory infection involving L. pneumophila.

Materials and methods: The agent was isolated from a sputum sample and Gram-stained smear was performed. Chocolate agar medium in which Legionella pneumophila can be isolated in a growth medium available in most microbiology laboratories under controlled °C in a 5% carbon dioxide, humid atmosphere. Legionella pneumophila was isolated in an unusual growth medium.

Results: Gram-stained smear obtained from sample showed that the specimen was suitable for culture. The agent grew on chocolate agar after 14 days of incubation at 35 ± 2 °C in a 5% carbon dioxide, humid atmosphere. Gram-negative rod bacteria was identified. MALDI-biotyper® and 16s rRNA gene sequencing identified the agent as Legionella pneumophila.

Conclusions: Legionella pneumophila can be isolated in a growth medium available in most microbiology laboratories under controlled conditions. This allows resource limited laboratories to obtain an isolate which might be important for further characterization and epidemiological studies.


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RHEUMATOID FACTOR AND ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODY, PIECES OF DIFFERENT PUZZLES?

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Introduction: Antibodies play an essential role in the evaluation of rheumatic diseases. (1) Testing for specific antibodies directed against the constant region of immunoglobulin G – rheumatoid factor (RF) – and antibodies to cyclic citrullinated peptides (anti-CCP) is part of the diagnosis and monitoring process of autoreactive diseases. (2) We aim to characterize patients with positivity for RF, anti-CCP or both markers simultaneously in respect to the presence of systemic disease with osteoarticular manifestations.
**Materials and Methods:** All RF and anti-CCP results between September 2007 and September 2017 were analysed. Patients were categorized as RF positive, anti-CCP positive and positivity for both markers. Patient data such as age, sex, and diagnosis were collected.

**Results:** Data from 405 patients was analysed. Isolated RF positivity was observed in 51% of patients, positivity for both markers was found in 38% and isolated anti-CCP was present in 11%. In all groups the majority of the patients were female and were in the sixth decade of life. In the group of patients with positivity for both markers, 96% had osteoarticular manifestations, in the isolated anti-CCP positivity group, it was observed in 82% and 63% in those with isolated RF positivity.

**Conclusions:** Patients with positivity for the two markers or isolated positivity for anti-CCP seem to have more osteoarticular involvement than those with RF positivity alone. This finding suggests that RF and anti-CCP might not reflect the same biologic entity.


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**P39**

**LOST IN CIRCULATION – THE TALE OF A CRITICAL RESULT**

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**Introduction:** Bacteria presence in peripheral blood films (PBF) is an unusual finding, normally associated with septicemia and consequently poor prognosis. The international council for standardization in hematology considers that this finding must be notified immediately to clinicians. The authors aim to present a case report of sepsis and disseminated intravascular coagulation (DIC) associated to *Streptococcus pneumoniae* and to discuss the eventual role of PBF review for diagnosis and rapid instatement of treatment.

**Material and Methods:** Case report of a 53-year-old man admitted to the emergency room with fever, shortness of breath, tachycardia and hypotension.

**Results:** At admittance, arterial blood gas showed metabolic acidosis. Further laboratory investigations showed hemoglobin 154 g/L, white cell count 4.0x10^9/L, neutrophils 3.4x10^9/L, platelets 19x10^9/L, C-reactive protein 15.8 mg/dL and procalcitonin 713 ng/mL. Prothrombin time was 28.5 s, fibrinogen 0.7g/L and D-dimers 111 ug/mL UEF. No signs of pulmonary embolism were detected. A review of the PB film revealed the presence of both intra- and extracellular diplococci and gram-positive diplococci were observed with Gram-stain. Detection of *Streptococcus pneumoniae* antigen in urine was positive. Altogether clinical and laboratory findings evidenced sepsis and DIC. Even though prompt intravenous antibiotic therapy was initiated, the patient died 10 hours after admission.

**Conclusions:** Presence of bacteria in the PBF is considered a critical result. This finding needs careful consideration due to the hypothesis of sample contamination but in our sample the presence of intracellular organisms was highly suggestive of septicemia. Although this can be the first available proof of septicemia PBF review isn’t a useful diagnostic tool due to lack of sensitivity when compared with blood culture, as microorganism concentrations of ≥10^5 CFU/mL would need to be present, making detection of bacteremia by routine PBF not possible in most cases. Regardless, in selected cases of suspected overwhelming bacteremia or in accidental PBF finding, reporting the presence of bacteria in PBF can provide a rapid preliminary diagnosis and improve therapeutic management.

**References**


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**P40**

**INDIVIDUALIZING THE COLISTINE DOSAGE REGIMEN IN RESISTANT MULTI-DRUG BACTERIAL INFECTIONS – PLASMA QUANTIFICATION BY LC-MS/MS IN PATIENT HEALTH IMPROVEMENT**

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**Introduction:** Due to its high morbidity and mortality levels the increasing prevalence of infections by multidrug resistant gram-negative bacteria (MDR-GNB) is currently a worldwide emerging clinical threat. Especially worrisome has been the identification of “superbugs” that are resistant to almost all contemporary antibiotics. The sodium colistimethate (CM) pro-drug is a last line treatment that is rapidly hydrolyzed in vivo into Colistin, a multicomponent lipopeptide containing predominantly Colistin A (CA) and Colistin B (CB) as the main active and
quantifiable metabolites. A safe and effective posology regimen requires the establishment of individualized therapeutic programs but the prodrug instability and the lack of a simple and selective methodology for quantifying the total Colistin \((CA + CB)\) in plasma turns to be a main difficulty for TDM in hospitalized patients. The authors present a liquid chromatography-mass spectrometry (LC-MS/MS) method suitable for the quantification of total Colistin in plasma.

**Methods and Materials:** A Waters Alliance 2695/Micromass Quattromicro system (LC-MS/MS) was used. The chromatographic separation was accomplished with a Mediterranean SEA 18 (Teknokroma) analytical column. Colistin sulfate, CM and gentamicin (internal standard-IS) were purchased from Sigma Aldrich. Convenient calibration curves and gradient mobile phases system were established. A positive electrospray ionization detection was used and the specific transitions \(m/z\) in the multiple reaction monitoring mode were \(585,3 > 576,7\) for CA, \(578,3 > 569,5\) for CB, \(590,5 > 581,5\) for CM and \(478,4 > 322,5\) for the IS.

**Results:** The required functional sensitivity and reproducibility for both CA and CB over a suitable dynamic range for TDM was achieved. CM degradation was controlled through a replica prepared and subjected to the same protocol. The runtime was fixed in 10 minutes with an injection volume of 50\(\mu\)L. All calibration curves displayed excellent linearity with \(r^2 > 0.998\) and controls revealed a CV\% < 10%.

**Conclusion:** This LC-MS/MS method proved to be specific, accurate and sensitive for quantification of plasma total colistine in plasma. It is simple, fast and appropriate to be adopted in the clinical laboratory routine preventing adverse effects and improving patient health condition in MDR-GNB.

**References:**

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A RARE CASE OF HEMORRHAGE ASSOCIATED WITH POLYCYTHEMIA VERA

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**Introduction:** Polycythemia vera (PV) is a chronic myeloproliferative neoplasm characterized by abnormal and overstated production of erythrocytes, leukocytes and platelets. The natural history of PV is characterized by an increased risk for thromboembolic complications however bleeding events may also occur.

**Case report:** A 76-year-old man with a diagnosis of PV since 2012, atrial fibrillation and hypertension began in March/2016 with gingival bleeding and some melena/hematochezia developing a microcytic hypochromic anemia. On that time he was taking hydroxyurea since 2012, rivaroxaban since March/2015.

He had had a myocardial infarction (1980), underwent bilateral inguinal hernia surgery (1966) and total knee replacement surgery (1999) without hemorrhagic complains or complications. He also had a family history of von Willebrand disease (vWD) (brother and nephews) but he had never been studied.

The hypothesis of therapy-related bleeding was initially admitted. An endoscopic study of the digestive tract was requested, which was normal, and he was also evaluated by Stomatología which did not found any problem.

In view of this evaluation and considering that the patient had a myeloproliferative neoplasm, the hypothesis of an acquired vWD was considered and finally diagnosed in April/2017.

**Discussion:** The trait of this case is the delay in diagnosis because the patient is under prohemorrhagic therapies. This case aims to alert us to the fact that we should consider the existence of a acquired vWD in patients with myeloproliferative neoplasms and with a recent history of mucocutaneous hemorrhages.

Acquired von Willebrand disease is a rare hemorrhagic diathesis, characterized by a lack of previous bleeding symptoms, negative familial history, and occurrence in a relatively older age. Most commonly, acquired vWD develops in the course of other conditions, such as lymphoproliferative, myeloproliferative, cardiovascular and autoimmune disorders.

Identification of the type of vWD of family members should be considered to clarify whether our patient is also a carrier of vWD which has been aggravated by the onset of acquired disease.

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sFlt-1/PlGF RATIO FOR THE PREDICTIVE DIAGNOSIS OF PREECLAMPSIA: BUDGET IMPACT ANALYSIS FROM THE PORTUGUESE HEALTHCARE PERSPECTIVE

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**Introduction:** Preeclampsia is a common medical problem found in pregnancy. Severe complications of the syndrome are uncommon but can be catastrophic, representing one of the leading causes of maternal and neonatal morbidity and mortality. The Elecsys sFlt-1/PIGF ratio is a new immunoassay recently available in Portugal for the short-term prediction and differential diagnosis of PE. The purpose of this study is to estimate the financial impact of introducing the sFlt-1/PIGF ratio for the evaluation of women with suspicion of preeclampsia in the Portuguese National Healthcare System (NHS).

**Methods:** A decision-tree model was used to estimate the budget impact of the introduction of the sFlt-1/PIGF ratio from the Portuguese NHS payer’s perspective. The model compares the management costs in the current clinical practice (“no test” scenario) vs. current diagnostic procedures plus the sFlt-1/PIGF ratio (“test” scenario). Clinical inputs have been derived primarily from PROGNOSIS study and from literature review. Resources and unit costs have been obtained from Portugal-specific sources.

**Results:** In the standard practice (no test), total costs associated with the management of women with suspicion of preeclampsia sum up to €9,863,264 (€1,160 per patient), compared to €9,781,194 (€1,150 per patient) with the introduction of the sFlt-1/PIGF ratio. This represents a net cost saving of €82,070 (€10 per patient) in the “test scenario” to the NHS. Savings are due to a reduction of false-positives, thereby decreasing the rate of unnecessary and overall hospitalizations.

**Conclusions:** The results of this budget impact study provide favourable economic evidence about the introduction of the Elecsys sFlt-1/PIGF test in the Portuguese NHS. Improved PE diagnosis leads to more accurate hospital admissions and interventions and allow a better allocation of resources. The generated savings more than offset the cost of the test.

**HbA1c DETERMINATION AND HEMOGLOBINOPATHY DETECTION WITH CAPILARY ELECTOPHORESIS – A CASE REPORT**

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**Introduction:** Glycated hemoglobin (HbA1c) determination with Capillary Electrophoresis provides a useful estimate of mean glycemia in patients with diabetes, directly related to risks for diabetes complications. An additional benefit of this method is the ability to detect the presence of hemoglobin variants.

In Portugal the global prevalence of hemoglobinopathies is 1-2%, however in the south of the country the prevalence can reach up to 5-10%.

**Case Report:** A 76-year-old male, with personal history of diabetes mellitus, chronic kidney disease and high blood pressure, attends to HESE for follow-up evaluation. The HbA1c determination by capillary electrophoresis reveals a trace of abnormal hemoglobin compatible whit HbS. None of the multiple previous HbA1c determinations of this patient showed this abnormality. Going through the patient history we realize that 15 days earlier he had received a blood transfusion. The Immunohemotherapy Laboratory was contacted to investigate the blood donor. Consequently the blood donor was summoned to perform an Hemoglobin Electrophoresis, that revealed 40,7% of HbS.

**Discussion/Conclusion:** Sickle cell trait is a relatively common hemoglobinopathy in the Portuguese population. According to World Health Organization (WHO) recommendations, the blood of such donors can be accepted when the minimum hemoglobin level for donation is reached. However this blood donor was contacted and a family study was recommended.

Since its implementation, the determination of HbA1c by this method has allowed the identification of several hemoglobinopathies previously unknown, being sickle cell traits, β-thalassemia traits and persistence of HbF the most commons.

**PRIMARY PLASMA CELL LEUKEMIA: A DIAGNOSTIC CHALLENGE**

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**Introduction:** Plasma Cell Leukemia (PCL) diagnosis requires more than 20% plasma cells (PC) in peripheral blood (PB) and an absolute PC count greater than $2 \times 10^9/L$. The clinical course is aggressive with short remissions and survival duration.

**Material and methods:** Review of clinical and laboratorial data of a patient with PCL.
**Case report:** A 61-year-old female with no relevant past medical history presented to her assistant doctor with hip and leg pain, weakness and anorexia. Physical examination was innocent. Complete blood count showed thrombocytopenia and leukocytosis with lymphocytosis. Serum biochemistry was normal except for elevated calcium, LDH and β2-microglobulin; there was no evidence of monoclonal gammopathy on protein electrophoresis, but serum immunofixation showed an IgG/lambda monoclonal band. Flow cytometry studies performed in our hospital revealed that 41% of blood lymphocytes were medium to large B cells with an aberrant phenotype, CD45-, CD19 +, CD20 -, CD79a +, CD38 +, CD138 -, CD56 -, CD117 -, and cytoplasmic lambda light chains +. Although atypical, it was suggestive of PC differentiation and was later supported by careful observation of PB smear which revealed 41% (3.8 × 10^9/L) of PC with immature characteristics: large cells with less condensed chromatin, high nuclear/cytoplasm ratio, some with nucleoli. Bone marrow morphology disclosed marked cellularity, with 84% of PC, mostly immature with some plasmablasts. Urinary free light chains revealed abnormal kappa/lambda free light chain ratio. Imaging studies disclosed multiple lytic lesions in the hip bone.

**Conclusion:** The workflow diagnosis in onco-hematology can be challenging and the final diagnosis results from the integration of clinical and laboratory data. We documented a primary PCL without evidence of extramedullary involvement. The diagnosis was made possible by ability of the pathologist to screen and recognize PC in the PB smear and to the capacity of flow cytometry to demonstrate B cell clonality, to identify PC and exclude other lymphoproliferative diseases.

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**TRACKING THE INTRA-HOSPITAL TRANSPORT: IMPLEMENTATION OF AN INNOVATIVE SYSTEM FOR THE PICKING OF SAMPLES FOR THE LABORATORY**

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**Introduction:** In healthcare systems, value is centered on patient care for which total safety and the best quality and financial management are required. In the clinical laboratory, and considering all daily time consuming activities, the arrival of large batches of untraceable samples and the occupation of professionals with non-adding value tasks (e.g. barcode reading or patient data registration prior to the analytical phase) are faithful illustrations of activities that compromise laboratory performance. In this regard we present the implementation of an innovative project that allows early management of laboratory requests from the entire hospital with the advantages of extending the traceability of samples, anticipate the registration of data and rationalizing the use of pneumatic transport lines.

**Methods and materials:** a Mini-Indexor® System (MIS, Maksense) connected to the hospital’s wireless network was incorporated in the mobile transport units that collect samples from the inpatient locations (IL). Simultaneously, MIS readers were placed in all pneumatic picking areas (PPA), including in the emergency room (ER). All sample barcodes were read (IL + PPA + ER) prior to transport to the laboratory and information sent to the laboratory information system (LIS) allowing the sample to be checked-in automatically and avoiding any additional step at the reception besides sample sorting. An evaluation was performed after one month of implementation and the processing time of a batch of EDTA samples from the Internal Medicine inpatient was compared to the time before MIS implementation.

**Results:** After one month, 37,743 samples were recorded by all MIS, 11,285 in mobile transport units and 26,458 in PPA and ER, without manual integration of petitions. Considering the EDTA samples, the first sample in batch have completed analysis within 27 minutes after reception in the laboratory. Before MIS, the time was highly variable, ranging from 31 to 52 minutes.

**Conclusions:** the implementation of MIS in the intra-hospital mobile transport units of samples and in all PPA proved to simplify the intra-laboratory pre-analytical process, reduce and standardize the turnaround time and enable improvement in future interventions based on documented evidence.

**References:**

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**CUTANEOUS MUCORMYCOSIS INFECTION – A CLINICAL CASE REPORT**

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**Background:** Mucormycosis, also known as zygomycosis, is an aggressive, extremely rare, life-threatening fungal infection, that can occur in immunocompromised patients.
The most common clinical presentation is rhinocerebral infection, although pulmonary, cutaneous, and gastrointestinal infections can also occur. Diagnosis is made when clinical and laboratorial criteria are present; the latter including direct microscopy and cultural demonstration of the etiological agent. Rhizopus spp are the most common organisms involved.

**Case presentation:** We describe a case of a 62-year-old male patient with renal chronic disease secondary to IgA nephropathy and multiple co-morbidities, that was transplanted in 05/09/2017. At nephrology follow-up, two months post-transplantation, the patient had surgical wound infection. Despite wide-spectrum antibiotic treatment he was readmitted in January without clinical resolution of the wound inflammatory signs. Analytically had a discrete increase in PCR (14.47 mg/L) and pancytopenia, renal function parameters were abnormal (creatinine 3.0 mg/dL, urea 155 mg/dL).

The surgical wound suppuration was collected and sent to the laboratory. In direct microscopy were observed numerous non-septate hyphae with branching at 90°. Fungal culture revealed the presence of *Lichtheimia corymbifera*.

The patient was then submitted to appropriate antifungal and surgical treatment.

**Discussion and Conclusion:** Mucormycosis is associated with long duration of hospitalization and 2-year shorter survival in patients with renal transplantation.

Surviving mucormycosis requires rapid diagnosis and aggressive multidisciplinary approach with medical and surgical treatment.

In this context, good quality representative clinical samples as well as rapid laboratory response are critical to the diagnosis. Since culture is less sensitive, microscopic demonstration of the characteristic broad hyphae in clinical specimens is the most crucial diagnostic tool and should never be ruled out.

References:


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**CMV PNEUMONIA – A CLINICAL CASE REPORT**

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**Background:** In patients infected with Human Immunodeficiency Virus (HIV), pneumonias by opportunistic microorganisms, related to the degree of depletion of CD4+ T cells, are common.

We present a case of an HIV infected patient with 57/mm3 CD4+ T lymphocytes, in which bronchoalveolar lavage (BAL) cells revealed typical morphology of cytomegalovirus (CMV) infection and were found to be essential for the clinical diagnosis.

**Clinical case:** A 38-year-old female, seeks medical attention in the Emergency Room (ER) of our hospital, complaining of cough and dyspnea with 5 months of evolution.

X-ray revealed bilateral interstitial infiltration. Computer Tomography (CT) confirmed signs of interstitial predominance. Assumed pneumonia and initiates antibiotic treatment, with reversion of symptoms.

A week later she returned to the ER because of recurrent symptoms. Analytically had an elevation of inflammatory parameters [C-Reactive Protein(CRP) 88.35 mg/L] and eosinophilia.

Although she was hospitalized with suspected Pulmonary Tuberculosis, *Mycobacterium tuberculosis* [Polymerase chain reaction (PCR)] and Ziehl Neelsen were negatives.

Further studies were made such as bronchofibroscopy with LBA collection for cyto-immunological study. Immunophenotyping of BAL: predominance of CD8+ T cells and 1% of CD4+ T cells. In the cytological examination rare large cells with cytoplasmic inclusions suggestive of “the owl’s eye cells” (CMV infection) were observed. Immunophenotyping of peripheral blood: CD4+T cells 57/mm3 and HIV viral load of 44,000 copies/mL.

After these results, serologies for CMV were performed (IgG 2575.0 IU/mL, IgM 2,610 IU/mL) and viral load of 413 copies/mL, confirming the infection.

**Discussion and Conclusion:** The cytological findings found in BAL are poorly sensitive, but very specific for this type of pneumonia. This finding allowed us to suspect the diagnosis, later confirmed by the serological study and the determination of the CMV viral load.

The LBA study is an easy-to-perform test, that requires few resources and allows identifying and characterizing cells and microorganisms, among other components. In this clinical case the cells observed were of special importance for study orientation and early diagnosis of CMV pneumonia before proceeding to further laboratory studies.

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1 McCabe RE; Diagnosis of pulmonary infections in immunocompromised patients; Med Clin North Am. 1988 Sep; 72(5):1067-89.
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PREDICTIVE VALUE OF ELECSYS® ANTI-HCV II ASSAY FOR SCREENING OF ACTIVE HCV INFECTION

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Introduction: Screening for active HCV infection has become a priority with the emergence of new therapeutic options. However, the association of anti-HCV antibodies with active infection, evidenced by the presence of viral RNA, is variable both by the sensitivity of the screening tests and by the persistence of the antibodies even after an eventual virus clearance.

Our hospital adopted in 2016 the Elecsys® anti-HCV II assay. The aim of this study is to determine, based on our two-year experience, the positive predictive value (PPV) of the test for screening of active HCV infection in our population.

Materials and Methods: PPV was determined using routine hospital samples tested between March 2016 and December 2017. The HCV RNA test results were searched for all samples with a cutoff ratio $\geq 0.90$. It was considered to exist active infection when there was an HCV RNA test positive in the two previous years or in the six months following the completion of the anti-HCV test. Cases where this information was not available were excluded. PPV was evaluated for cutoff ratios of 0.90, 10.0, 20.0, 30.0 and 40.0.

Results: 20208 sera were tested, of which 622 (3.08%) were reactive. 133 were excluded due to lack of information. Of the remaining 489, 414 (84.7%) had a positive RNA test in the two previous years or in the six months following the anti-HCV test. The probability of a patient with active infection having an undetectable viral load is quite low, which limits the associated error (Scott 2006).

PPV for cutoff ratio values of 0.90, 10.0, 20.0, 30.0 and 40.0 was, respectively, 84.7%, 88.4%, 90.9%, 92.0% and 94.5%. Published studies differ greatly in cutoff values needed for PPV $\geq 95\%$, ranging from 20.0 (Yang 2013) to 150.0 (Baumann 2011). Setting a threshold requires more investigation.

Conclusions: PPV of Elecsys® anti-HCV II assay in our population is good compared to published data. However, it is not possible to set a threshold that waives the use of a confirmation test.

References
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A CRYPTOCOCCUS THAT LIVES UP TO ITS NAME: A CASE REPORT ON CRYPTOCOCCAL MENINGITIS

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Introduction: Cryptococcal meningitis (CM) is a life-threatening fungal disease affecting both immunosuppressed and immunocompetent individuals. Most serious infections usually develop in patients with AIDS, undergoing organ transplantation, with reticuloendothelial malignancy, undergoing corticosteroid treatment, and sarcoidosis. Cryptococcus presumptive diagnosis is made by India ink test, culture and identification for diagnosis confirmation.

Case Report: A 47 year-old caucasian male patient with a clinical history of hypogammaglobulinemia, high blood pressure, LCAT deficiency (with membranous glomerulopathy [taking methylprednisolone 24/mg/day], hemolytic anaemia, bilateral cornean opacity), presented to Abrantes Emergency Department (ED) with fever and syncope without prodromes. He had already gone to the same ED 2 days before for occipital headache, cervicalgy, some mental confusion, and upper limbs paresthesia. A brain CT scan (no acute lesions) and a cervical CT scan (some degree of stenosis) were performed. In the latter episode, the clinical examination wasn't conclusive. Laboratory tests revealed leukocytosis with neutrophilia, lymphopenia, anaemia (haemoglobin 8.1 g/dL), 333000 platelets, creatinine 2.2; CRP 2.26 mg/dL, and proteinuria. After that he had a lumbar puncture and was hospitalized. CRF analysis: 75% PMN leucocytes (81/mm³ total) and the presence of morphologically small typical yeasts that were negative to the India ink test. After overnight incubation culture, yeasts were identified: poor Vitek2 identification as Candida sp. As the presence of Cryptococcus was highly suspicious, an external laboratory ID was performed on MALDI-TOF: 99,9% Cryptococcus neoformans. The patient started liposomal amphotericin B. A week later the lumbar puncture was repeated,
but then the India ink test was positive for Cryptococcus with a more typical morphology. His clinical situation improved and was discharged after 26 days.

**Conclusion:** This case report brings relevance to the difficulty of presumptive diagnosis of Cryptococcus neoformans, particularly its morphological variations and the lack of sensitivity for the India ink test on non-HIV patients (just 50%).

**References:**

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**ROLE OF PRE-ANALYTICAL CONDITIONS IN THE EVALUATION OF FREE PLASMA METANE-PHRINES BY LC-MS / MS**

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**Introduction:** Pheochromocytomas are rare catecholamine-producing tumors that arise from adrenal tissue. Plasma free metanephrines (normetanephrine (NM) and metanephrine (M)) are final metabolites able to be quantified by tandem mass chromatography (LC-MS / MS). Several pre-analytical conditions interfere in the results as drugs, diet and resting state. We retrospectively evaluated the results of free plasma metanephrines analysed in our laboratory and correlated them with the pre-analytical conditions.

**Material and Methods:** The results were collected anonymously from laboratory middleware database during an 18 months period and organized according to three groups: normal concentration (M < 60 pg/ml and NM < 120 pg/ml); moderately elevated (M: 60 – 240 pg/ml; NM: 120 – 400 pg/ml) and elevated (M > 240pg/ml; NM > 400 pg/ml). Age, sex and diagnosis were also taken into account. Data were analyzed with SPSS software.

**Results:** During the 18 months were performed 561 analysis from 472 patients. 41% of the patients were men and 59% were women. The mean age of men was 54.5 ± 17.3 (min: 11 years – max: 93 years) and the mean age of women was 54.0 ± 16.7 (min: 8 years – max: 95 years). Metanephrine plasma concentration was below 60 pg/ml in 92.6% of the patients, between 60 pg/ml and 240 pg/ml in 4.44% of the patients and above 240 pg/ml in 3% of the patients. Nor-metanephrine, was below 120 pg/ml in 87.9% of the patients, between 120 pg/ml and 400 pg/ml in 9.7% of the patients and above 400 pg/ml in 2.3% of the patients. Due to the suspicion of pre-analytical errors, 32 out of 472 patients (6.8%) were hospitalized to repeat the analysis. 13 of these patients upon repetition of the determinations, showed normal values for both of the metanephrines.

**Conclusions:** The pre-analytical conditions are determinant to achieve a correct evaluation of normetanephrine and metanephrine. The use of inappropriate sampling conditions leads to the performance of unnecessary image tests, to the raise of anxiety and stress in the patients and raises health care costs.

**References:**

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**CRP VS CRP OF HIGH SENSITIVITY: COMPARISON BETWEEN TWO METHODS**

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**Introduction:** CRP is a acute phase protein and its concentration change faster and more widely than the other acute phase proteins, which make its determination very useful in a great number of clinical conditions.

**Method:** 98 samples of serum were used to determinate the value of CRP, using the method currently in use and the method of high sensitivity (hs) for the determination of CRP hs, and a comparison study was made. The technic for the determination of CRP-hs is Immunoturbidimetry and, for the method in use for CRP it is Immuno-kinetic fixed-point test.
Results: The minimum value of CRP obtained was 0.9 mg/dL and the minimum value of CRP-hs was 0.01 mg/dL. Spearman's correlation coefficient (r) for the overall performance of the two methods was 0.986.

In relation to the number of dilutions of the samples, it was verified that the method of CRP-hs allows to make fewer dilutions in relation to the method in use.

Conclusions: Based on this study, it was verified that there is a good agreement between CRP values obtained by the 2 methods. It was found that the high sensitivity technique for CRP determination has a lower detection threshold than the other method. The method of CRP-hs allows faster determinations, without having to carry out so many dilutions of the sample, which is of great importance in laboratory practice.

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PURE RED CELL APLASIA SECONDARY TO PARVOVIRUS B19 INFECTION – A CASE REPORT

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Introduction: Acquired pure red cell aplasia (PRCA) is a rare condition of profound anemia, characterized by a severe reduction in the number of reticulocytes in the peripheral blood (<1%) and the virtual absence of erythroid precursors in the bone marrow (<0.5%). All other cell lineages are present and appear morphologically normal. PRCA in the adult is most often idiopathic, although a number of underlying causes can exist, such as lymphocytic leukemia, lymphoproliferative disorders, myelodysplastic syndrome, thymoma, auto-immune diseases, drugs or viral infections as by Parvovirus B19.

Case Report: We present a clinical case of a 76-year-old male with history of hypertension, dyslipidemia, type 2 diabetes mellitus, surgically treated unspecified malignant bladder neoplasm and soft tissue sarcoma on the right leg (treated with surgery and chemoradiotherapy), admitted to the emergency department complaining of weakness, dizziness and dyspnea for 2 days. He was hemodynamically stable, tachypneic with an oxygen saturation of 99% on air, along with skin and mucosal pallor. Analytically, he had a respiratory alkalemia with hyperlactatemia (2.4 mmol/L), normocytic and normochromic anemia (hemoglobin 4.7 g/dL, MCV 109.8 fl, MCH 38.1 pg) and reticulocytopenia (0.8%). 2 units of packed red blood cells were given and the patient was hospitalized for further investigation. During the hospitalization, a gastrointestinal workup (esophagogastro-duodenoscopy and colonoscopy) in search of a source of acute bleeding was performed, without revealing it. Ferritin levels were high (833 ng/mL); both serum folate (6.98 ng/mL) and vitamin B12 (472 pg/mL) concentrations were within the normal range and serum erythropoietin was very high (>797 mU/mL). A CT-Scan was performed, which did not reveal any lesions compatible with metastasis. A bone marrow aspiration was performed. The morphological aspects were consistent with PRCA. This motivated the reflex testing for Parvovirus B19 DNA in the bone marrow aspirate. Using Real Time PCR, PVB19 DNA was detected.

Discussion: The authors intend to emphasize the importance of the diagnosis of pure red cell aplasia when a patient presents with isolated anemia associated with reticulocytopenia and the consideration of parvovirus B19 infection as a cause, which may be forgotten.


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AN EXUBERANT CASE OF URIC GOUT IN A PACIENT DIAGNOSED WITH CHRONIC LYMPHOCYTIC LEUKAEMIA: A CASE REPORT

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Introduction: Chronic Lymphocytic Leukemia (CLL) is a monoclonal disorder characterized by an accumulation of functionally incompetent CD5+ B-lymphocytes. Metabolic disorders are possible complications in onco-hematological patients, with hyperuricemia being the most common. These conditions can be pre-disposed or worsened by pre-existing comorbidities, such as kidney or cardiovascular dysfunction. Even though successful treatment of the underlying tumor often improves metabolic disorders, these conditions often worsen prognosis and decrease live quality. Thus, an early diagnosis and an effective treatment are important.
Case Report: An 88-year-old female diagnosed with chronic kidney disease (CKD) and chronic lymphocytic leukaemia (CLL) being followed in Haematological consultation at Centro Hospitalar de Leiria (CHL) since 2008, presents a history of multiple attendances of the Urgency Service and recent admissions in the Intermediate Care Unit of CHL, where she was treated with antibiotics for a soft tissue infection of the right foot, complicated with an abscess and an acute uric gout episode of the same member. Shortly after discharge, she returned to the Urgency Service reporting acute pain and swelling of the right foot. The physical exam showed inflammatory signs of the foot, cyanosis of the 1st and 2nd toes and spontaneous drainage of a soft amorphous mass on the 1st metatarsofalangic joint. Analytically she presented elevated inflammatory parameters (PCR 175mg/L; Leuc. 84700/μL, Neut. 16000/μL, Linf. 66600/μL) and hyperuricemia (463μmol/L). A sample of the mass was collected and sent to both Anatomical Pathology (AP) and Clinical Pathology (CP) services for analysis.

Materials and Methods: A small quantity of the sample was suspended in physiological saline, placed on a slide and observed in the optical microscope, at 10x and 40x magnification.

Results: Multiple birefringent, needle-shaped structures, compatible with uric acid crystals were observed. The analysis performed at the AP service corroborated these results.

Conclusion: The collaboration between the AP and CP services provided a quick response, aiding the clinician in establishing a correct diagnosis of uric tophi and prescribing a correct treatment, dispensing the patient from doing another cycle of antibiotics.

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CRYOFIBRINOGENEMIA, A CASE REPORT

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Introduction: Cryofibrinogenemia refers to the formation of a cryoprecipitate in the plasma. It differs from cryoglobulinemia by the absence of cryoprecipitate in serum. It is considered a rare disorder, being detected in healthy individuals (primary or essential cryofibrinogenemia) and in patients with infections, autoimmune diseases and malignancies (secondary cryofibrinogenemia).

Materials and methods: We report a case of a 73-year-old man referred to internal medicine for the study of an infiltrative process in the posterior mediastinum and extensive cutaneous lesions of the lower limbs.

Results: The analytical study revealed multiple abnormalities. Serum IgM/Kappa monoclonal gammopathy, moderate positivity for Rheumatoid Factor, high positivity for anti-neutrophil cytoplasmatic autoantibodies (anti-PR3/C-ANCA) and formation of plasma cryoprecipitate were detected. Immunotyping of the cryoprecipitate was compatible with the presence of fibrinogen/fibrin, fibronectin and another IgG/Kappa monoclonal gammopathy. Cutaneous biopsy of the lower limbs revealed morphological features suggestive of leukocytoclastic vasculitis. Two unsuccessful attempts at biopsy of the posterior mediastinal lesions detected in imaging tests were performed. All lesions and complaints of the patient regressed after the start of steroid therapy.

Conclusions: Despite being considered a simple and inexpensive test, the screening for cryoproteins (cryoglobulins and cryofibrinogen) poses some technical challenges (in the detection and characterization of proteins) as well as doubts in its clinical interpretation. Cryofibrinogenemia in this patient was considered secondary to autoimmune disease. Despite a clear clinical improvement with corticotherapy, the atypical coexistence of two monoclonal gammopathies demands clinical, laboratory and imagiological surveillance of this patient for the early detection of malignancie, namely a lymphoproliferative syndrome.

References

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USE OF NEW TOOLS TO SUPPORT LABORATORY MANAGEMENT - THE POWER OF ARGUMENTS

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Introduction: The use of the enormous potential of data of the Clinical Pathology Services (SPC) is something that many already know to take sides. There are a number of clinical services that rely exclusively on this initial information to select cases or sets of data to be subsequently
supplemented with other clinical records and hence contribute to the wider publications where, fortunately, due opened up real motorways in the vastness of existing data.

Nowadays, the Laboratory Information Systems (SIL) is one of the pillars of medical practice, both for the ease of data collection and for its broader use of monitoring and allowing analysis of the prescription process.

On this basis, we have tried to place one of the tools of our SIL - Control Panel, in the service of the monitoring of electronic prescription according to a DGS standard.

**Materials and methods:** Control panel of the laboratory information system of the Clinical Pathology Service. Systematic evaluation of electronic prescription records addressed over time to a DGS NOC. Performance indicator adapted to the NOC.

**Results:** The results scattered over three years are presented according to the formatting of the control panel. In a graphical way, the implementation of training and the provision of the new form of electronic prescription adapted to the NOC of the DGS is quickly followed. It can be observed that with the passage of time there is a loss of focus regarding directed electronic prescription. The results also show that there was learning regarding NOC because with the abandonment of the directed prescription there was no reverence of the appropriate prescription.

**Conclusions:** The use of the new management tools allows to monitor, both in real time and retrospectively, the systematic use of these tools contribute to the positive argumentation within the prescribing community and to increase the argumentation with the hospital administrations.

**P56**

**LIGHT CHAIN MULTIPLE MELOMA – A CASE REPORT**

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**Introduction:** Multiple myeloma is a malignant hematological disease resulting from tumor proliferation of a clone of plasma cells, with invasion of bone marrow and secretion of a serum paraprotein. It is a rare disease that accounts for about 1% of all cancers and 10% of all malignant blood disorders. We report the case of a 66-year-old patient, native of Guinea-Bissau, where he was hospitalized for 2 months for lumbo-sacral bone pain and fatigue. He was then advised to resort to the Portuguese hospital services for etiological investigation. At admittance to the emergency room of our center, the patient presented with anemia (52 g/L), renal injury (creatinine 4.22 mg/dL), proteinuria (+++, ≈ 300 mg/dL) and hypocalcemia (15 mg/dL).

**Materials and methods:** Given the strong suspicion of multiple myeloma, the patient was hospitalized in the internal medicine department and the clinical pathology service contributed to the diagnosis through the morphological evaluation of peripheral blood, bone marrow and immuno-chemistry parameters.

**Results:** Morphological evaluation of peripheral blood showed some lymphocytes with nuclear dysmorphism; serum protein electrophoresis with a discrete peak in the beta-gamma transition; urinary proteinogram with monoclonal peak; serum and urinary immunofixation with monoclonal band of free kappa light chains, compatible with Bence-Jones protein; determination of intact immunoglobulins revealed immunoparesis of the IgA and IgM; serum free light chains: kappa 10211 mg/L, lambda 10.3 mg/L and k/l ratio 991; myelogram with infiltration by atypical cells of the plasmocytic lineage (50%); immunophenotyping of medullary blood with 100% monoclonal plasma cells with aberrant phenotype; β2-microglobulin 16 μg/mL; LDH 192 IU/L; albumin 3.1 g/dL.

**Conclusion:** The diagnostic work-up pointed to light chain multiple myeloma (LCMM) of the kappa type, stage III-ISS. This entity represents about 9% of all myelomas. Its diagnosis can be difficult either if there is too little secretion of paraprotein occurring or if the clinical presentation does not point to this entity. In this particular case, it’s noteworthy that the absence of an obvious monoclonal peak in the serum protein electrophoresis does not correlate with the severity/activity of the disease. As slight changes of the proteinogram may be ignored, we reiterate the importance of monitoring and detecting the monoclonal component by other analytical methods, especially in the case of non-secretory or free light chains MM. In these cases, the free light chain assays may be more sensitive.

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**ALBUMINURIA AND HYPERCALCIURIA IN DIABETES MELLITUS - IS THERE A CORRELATION?**

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**Introduction:** Diabetes mellitus (DM) is currently the leading cause of chronic renal failure, and the presence of albuminuria the first manifestation of kidney disease. Many DM patients have hypercalciuria, which can contribute to osteodystrophy and to the consequent increased risk of bone fractures. The pathophysiological mechanism leading to hypercalciuria in Diabetes is not well established, and may yet be
associated with changes in tubular reabsorption of calcium. This study aimed to assess the impact of albuminuria and renal function, on Ca excretion in DM patients.

**Material and methods:** Patients (n = 197) with DM and without DM (NDM) were evaluated by standard biochemical methods for serum Ca and creatinine, and urinary albumin, Ca and creatinine. Ca excretion fraction, urinary albumin-to-creatinine ratio (ACR) and glomerular filtration rate (GFR) were computed. Each DM/NDM group was further stratified according to: albuminuria (> 10 mg/L), ACR (> 30 mg/g) or GFR (> = 60 mL/min/1.73). A sub-population (n = 77) was assessed for phosphaturia. Data were compared and differences considered significant for p < 0.05.

**Results:** Biochemical data shows reduction (p < 0.05) of creatinuria and phosphaturia and increase (p < 0.05) in ACR and in fraction of Ca excretion in DM compared with NDM. When groups are stratified by albuminuria, the Ca excretion fraction is identical between both DM groups and between groups with albuminuria but reduces in the NDM group with albuminuria (p < 0.05), which does not occur in the DM group with albuminuria. The same pattern of Ca excretion is observed both when patients are stratified by ACR or by GFR. Phosphaturia is similar in non-albuminuria groups but in its presence there is a reduction of phosphaturia in DM group (p < 0.05). When groups are stratified by ACR, DM patients show reduction (p < 0.05) of phosphaturia even when renal function is still uncompromised (<30 ACR). When GFR is compromised the phosphaturia decreases in both DM and NDM for similar values.

**Conclusions:** This study confirms the presence of hypercalciuria in DM patients and suggests a non-association with the renal function impairment, demonstrated by the albuminuria and the decrease of GFR. To further clarify the cause of hypercalciuria in DM other serum parameters such as Ca, phosphate, Mg, PTH and vitamin D need to be evaluated.

**P58**

**COMPARATIVE ANALYSIS OF AN IMMUNOCHEMISTRY METHOD WITH RADIOIMMUNOASSAY FOR THE MEASUREMENT OF SERUM IGF1**

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**Introduction:** Insulin-like growth factor-I (IGF-I) is a hormone that functions as the major mediator of growth hormone (GH) signaling for somatic growth. IGF1 works as a better serum marker for GH activity, since the latter has very short half-life and is produced in peaks by the hypothalamus.

In clinical practice IGF1 measurement has particular use in the diagnosis of disorders associated with growth hormone deficiency (such as slow growth and delayed development in children) or over production which can lead to gigantism or acromegaly.

This research was conducted to assess the quality of the results given by the reformulated method developed for the IMMULITE 2000® (Siemens), for the determination of serum IGF1 concentration. As such and since no reference method is defined, we performed a comparison study with the Radioimmunoassay (RIA) method currently used in our laboratory to assess for the accuracy; the method’s precision was also tested.

**Materials and Methods:** Precision assessment was performed by ANOVA test. The same sample was tested three times, twice a day (in the morning and afternoon) for 5 days. Imprecision was accessed by variance analysis.

For the comparison analysis 82 samples were analyzed using both methods and statistical comparison of the data was conducted. Samples used were from patients of our Hospital whose IGF1 measurement was requested by clinicians.

Samples were sent to the lab and an aliquot was frozen (at -20 ºC) for future determination, according to the lab work flow. When the number of samples was sufficient measurements were made on the same day using both methods.

For the RIA method the IGF-I RIA-CT kit (Mediagnost, Germany) was used and the measurements were made using Wizard²™ Gamma Counter.

**Results:** We found a repeatability of 3.9%, an intermediate imprecision of 3.6% and an intralaboratory imprecision of 5.3%, which was higher than the imprecision reported by the product manufacturer.

In regards to the comparative analyses, we found a difference of -73 ng/mL (-88.2 to -59) in the difference plot analyses. In the Passing-Bablok regression we found a regression equation with slope value of 0.644 (0.619 to 0.665) and an intercept value of 5.346 (2.622 to 9.011).

**Conclusion:** Results found point to a systematic error, particularly as the values of the concentrations measured increase.* These abstracts have been reproduced directly from the material supplied by the authors, without editorial alteration by the staff of this Journal. Insufficiencies of preparation, grammar, spelling, style, syntax and usage are the authors’ responsibility.