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EVALUATION OF LETITEST KPC K-SET RAPID IMMUNOCHROMATOGRAFIC TEST FOR DETECTION OF KPC CARBAPENEMASE

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Introduction: Carbapenemase production is a common resistance mechanism against Carbapenems encountered in Enterobacteriaceae.
The presence of Carbapenemase is associated with high level resistance to all β-lactam antibiotics, but also, to a lesser extent, with concomitant resistance to other antibiotic groups. Therefore, there is epidemiologic and clinical importance in rapidly identifying their presence.
In our hospital, the most common Carbapenemase identified is KPC, having already been associated with outbreaks.
As such, we developed the need to confirm their presence in Enterobactereaceae strains that were phenotypically resistant to Carbapenems.
The method of confirmation initially used was real-time PCR, which allowed for a quick detection and an earlier isolation of patients.
But real-time PCR requires a dedicated environment, skilled people and is expensive. Could we do better?
Our aim was to evaluate a rapid immunochromatografic test "Letitest KPC K-SeT® (Leti diagnosis)", and compare it with the real-time PCR test already in use in our Laboratory "Xpert® Carba-R (Cepheid)".
Material and Methods: 19 Enterobacteriaceae strains, isolated from patient samples, that had been classified as Carbapenem resistant by phenotypic methods (VITEK 2® automated analyzer, Biomérieux), were evaluated for the production of KPC Carbapenemase using both a rapid immunochromatografic test Letitest KPC K-SeT® and the real-time PCR test Xpert® Carba-R, and their results were compared.
1 Carbapenem susceptible Enterobacteriaceae strain was evaluated as well, using Letitest KPC, to access the test's capability of producing negative results.
Results: 19 Carbapenem resistant strains were evaluated. All of the 19 strains tested positive for KPC Carbapenemase, both by Letitest KPC and by real-time PCR. The agreement between the two methodologies was 100%.
The 1 Carbapenem susceptible strain tested negative by the Letitest, as expected.
Conclusion: Letitest KPC proved to be a fast, reliable and efficient method of detection of KPC in Enterobacteriaceae. Moreover, it is also more economical than real-time PCR.
This comparison allowed us to implement Letitest KPC in our Laboratory routine, which in turn led to production of faster and equally reliable results, at lower cost, with the associated clinical benefits.

CEREBRAL CREATINE DEFICIENCY SYNDROMES: NON DERIVATIZED QUANTIFICATION OF GUANIDINE ACETIC ACID AND CREATINE BY LIQUID CROMATOGRAPHY TANDEM MASS SPECTOMETRY

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Introduction: Cerebral creatine (CR) deficiency syndromes (CCDS) are inborn errors of metabolism that include two CR biosynthesis disorders and a CR transporter deficiency (CRTD). Guanidinoacetate methyltransferase (GAMT) deficiency and L-arginine:glycine amidinotransferase (AGAT) deficiency are autosomal recessive disorders while CRTD is a X-linked disorder. Global development delay, seizures, intellectually disability with speech delay or even speech absence, movement and behavior disorders are common signs and symptoms. An early diagnosis and treatment are crucial to prevent clinical manifestations of GAMT and AGAT deficiency. Clinical suspicious ranges from infancy to adulthood and diagnostic relies on guanidinoacetate (GAA), CR and creatinine quantification in urine or plasma, followed by gene sequencing or enzymatic activity in cultured fibroblasts or lymphoblast. The authors present an in-house liquid chromatography tandem mass spectrometry (LC-MS/MS) non-derivatized method for simultaneous quantification of CR and GAA in urine.
Methods and Materials: The LC-MS/MS non-derivatized method was developed using an Altantis T3 5µm 2,1x150 mm analytical column (Waters), with positive electrospray ionization detection and suitable gradient mobile phase system. GAA and CR standards were purchased from Sigma-Aldrich and respective deuterated compounds from Cluzeau Info Labo. Specific m/z transitions in multiple reaction monitoring mode were: 132 > 90 (CR), 118 > 76 (GAA), 135 > 93 (CR-D3), 120 > 78 (GAA-D2). Calibration curves ranged from 114 – 7626 µmol/L (CR) and 60 – 4269 µmol/L (GAA). A sample volume of 100 µL was used for a 10 µL injection.
Results: The method achieved the required functional sensitivity and reproducibility, for both GAA and CR over a suitable diagnostic range. Reproducibility was excellent for both compounds (CV < 10 %) and all calibration curves displayed excellent linearity, with r2> 0.9990. Runtime was fixed in 4 minutes.
Conclusions: The LC-MS/MS here presented provides a simple, rapid, precise, and specific method for the quantification of CR and GAA in urine improving laboratory's management of inborn errors of metabolism, allowing early diagnostic and consequent institution of therapeutics, essential in pediatrics age.

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HE4, CA125 AND ROMA PERFORMANCE IN THE EVALUATION OF OVARIAN MASSES – A 5 YEARS STUDY

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Introduction: Ovarian cancer is the 5th death cause in women worldwide. It affects mainly women in postmenopausal with a peak between 55 and 65 years. The pre-surgical evaluation of the pelvic mass plays an important role in the development and outcome of the disease. 90% of the ovarian tumors are epithelial and the serous type is the most frequent (50-60%). Borderline tumors (BT) are a particular group (10%) of epithelial tumors that mainly affect premenopausal women. They present low grade malignancy, however 20-25% of cases disseminate through out ovary.
Objective: To evaluate the performance of ovarian tumor markers (CA 125, HE4) and Risk of Ovarian Malignancy Algorithm (ROMA) in the pre-surgical differentiation between malignant and benign adnexal masses.
Methods and Material: CA 125 and HE4 were determined in pre-surgical serum samples from women with adnexal masses, between August 2011 and December 2016. Levels of both CA125 and HE4 were measured by chemiluminescence in a fully automated ARCHITECT instrument (Abott Diagnostics, IL, USA). ROMA was calculated with Clinidata XXI (Maxdata Healthcare Solutions, Carregado, Portugal). All samples with creatinine > 1,1 mg/dL were excluded. The histological type of tumor was obtained by histopathological evaluation.
Results: Based in the histopathological evaluation, the sample (N = 640) was divided in 3 groups:
A: All malignant tumors (N = 264); B: Malignant Tumors, except BT (N = 213); C: BT (N = 51) and they were compared with benign disease (N = 376).
HE4 obtained a Sensibility (S) of 68%, 80% and 20% in groups A, B and C respectively with a specificity (E) of 98%. AUC was 0,891; 0,941; 0,680 for groups A, B and C.
CA125 obtained a S of 86%, 92% and 57% in groups A, B and C respectively with an E of 80%. AUC was 0,906; 0,946; 0,739 for groups A, B and C.
ROMA obtained a S of 86%, 93% and 55% in groups A, B and C respectively with an E of 83%. AUC was 0,901; 0,951; 0,695 for groups A, B and C.
Conclusions: HE4 is the most specific marker for the diagnosis of ovarian malignancy in all groups. CA125 is the most sensitive marker in the evaluation of ovarian masses regardless of the histopathological evaluation. The combination of both markers (ROMA) was the best tool to assess the risk of ovarian malignancy. All the markers showed poor outcomes evaluating BT, probably due to the behavior of this tumors.

DETECTION OF MINORITY CLONES IN ACUTE LYMPHOBLASTIC LEUKAEMIA BY FISH ANALYSIS IN SEPARATED CELLS – IMPACT ON PROGNOSIS AND THERAPEUTIC DECISION MAKING (CLINICAL CASE)

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Introduction: Acute lymphoblastic leukaemia (ALL) is one of the most common neoplastic diseases within paediatric groups. Its diagnostic is usually rather simple, established by the observation of ≥20% lymphoblasts in the bone marrow or peripheral blood. Afterwards, it's imperative to proceed to its classification according to the World Health Organization (WHO) standards, using molecular genetics and phenotypical data, seeing that the several subtypes produce different prognosis, and thus distinct therapy options.
Clinical case: We describe the case of a male adolescent, 16 years old, which reported bloody diarrhoea and fatigue with 1 week of evolution, mentioning an isolated episode of fever. The physical exam was negative to adenopathies or any other changes besides skin pallor. Laboratory results showed leucocytosis (44.500/μL) with 91% blasts, which immunophenotypical studies classified as ALL B lineage. Additionally, the cerebrospinal fluid was negative to the presence of blasts and the image studies showed no masses in the mediastinum. Molecular biology and FISH hybridization studies (of the blasts) detected only genetic rearrangements of KMT2A (Lysine[K]-specific MethylTransferase 2A) in 6% of the blasts, not having been found t(4;11) KMT2A-MLLT2; t(9;11) KMT2A-MLLT4; t(6;11) KMT2A-MLLT1 or t(11;19) KMT2A-MLLT1.
Discussion/Conclusion: We face ourselves with a situation where the FISH study shows genetic rearrangement in 6% of the blasts. Considering that the technique cut-off in our laboratory is 2%, and being that the ALL-B with KMT2A rearrangements is, according to the 2016 WHO classification, a leukaemia subtype associated to a worse prognosis and a high rate of relapse, we question ourselves if the results gathered in these small clone of 6% blasts should be used for the classification and prognosis or if it should be based on the significantly bigger 94% blast clone of cells with no KMT2A rearrangement.
This question is of particular relevance since the treatment of leukaemia with a grave prognosis is different than the treatment offered to someone with a standard prognosis, something where the laboratory report has a tremendous impact and will greatly affect the child's therapy.

THE RELATIONSHIP BETWEEN PARENT'S EDUCATIONAL LEVELS WITH OFFSPRING'S WBCS AND ITS SUBTYPES ON EPITEEN, AN URBAN YOUTH COHORT

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Introduction: The immune system's regulation, seems to be influenced by several social determinants, such as parent's educational levels (PEL), although the underlying physiological mechanisms are still far from being clarified. In effect, despite the lower PEL have been described to be positively associated with offspring's immune markers such as, CRP, IL-6, Fibrinogen and MCP-1 even when adjusted for potential confounders such as BMI and physical activity, the assessment with cell-mediated immune mediators might add new insights for the pathophysiological mechanisms linking PEL with immune system's regulation in the early stages of life.
Material and Methods: Participants were part of the prospective Epiteen cohort during first evaluation, at 13 years old (n = 1213, 53.1% female). PEL data were collected using self-administrated questionnaires. Blood counts were assessed using an automated blood counter Sysmex ® XE-5000. In order to assess the association between PEL and hematological parameters regression models were fitted to estimate beta coefficients (β) and 95% CI stratified by median hs-CRP levels.
Results: PEL were negatively associated with WBCs (β = -0.03, 95% CI:-0.05,0.00, p = 0.031, for CRP ≤ 0.3mg/L; β =-0.06, 95% CI:-0.09,-0.02, p = 0.001, for CRP > 0.3mg/L) and Neutrophils (β = -0.23, 95% CI: -0.40,-0.06, p = 0.007, for CRP ≤ 0.3mg/L; β =-0.23, 95% CI:-0.44,-0.02, p = 0.030, for CRP > 0.3mg/L) and positively associated with lymphocytes (β = 0.20, 95% CI: 0.05, 0.35, p = 0.010, for CRP ≤ 0.3 mg/L). These results remained significant when adjusted for sex, BMI and chronic disease. No statistically significant association were reported for Monocytes, Basophils and Eosinophils.
Conclusion: In this study, PEL were negatively associated with offspring's WBCs and neutrophils, which are involved in first line of immune system activation, and positively associated with lymphocytes, the regulatory arm of the immune system. This results piles new evidence that a lower PEL seems to promote offspring's immune system upregulation and, consequently, trigger an early increase in systemic low-grade inflammation, and therefore, these adolescents might be at a higher risk of cardiovascular disease in adulthood.

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MACRO TSH AND PREGNANCY: SOMETHING TO TALK ABOUT

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Abstract: During the last four decades, there have been considerable advances in the efficacy and precision of serum thyroid function testing. The development of the third and fourth generation assays for the measurement of serum thyroid stimulating hormone (TSH, thyrotropin) and the log-linear relationship with free thyroxine (T4), established TSH as the hallmark of thyroid function testing.
Discordance between TSH and Free T3 and FreeT4 (FT4) results, without clinical signs or symptoms of hypothyroidism are considered as having an immunological interference by heterophile antibodies or that we are in the presence of a form of macro-TSH consisting of an immune complex bound to the anti-TSH autoantibody.
Analytic-antibody complexes are a well-known cause of clinical misinterpretation of endocrine results. This is especially important during pregnancy, when the thyroid is under considerable additional pregnancy-related demands requiring significant maternal physiological changes. [1]
Methods / results: We used Protein G Agarose Gel (Roche) and polyethylene glycol precipitation test (PEG) methods, to do the separation of TSH from the complex. We examined a case of a 33 year old pregnant woman with a mild elevated thyroid-stimulating hormone (TSH) level [5.850mUI/L], measured by a chemiluminescence immunoassay (Advia Centaur XP, Siemens), but with no specific symptoms of hypothyroidism. Levels of free T3 and free T4 were normal [3.51pg/mL and 0.99ng/dL, respectively] and the autoimmune antibodies (anti-thyroglobulin and anti-peroxidase) were negative. After PEG precipitation the concentration of TSH was measured and the contribution of macro-TSH to the total TSH concentration was calculated. The recovery rate was 56% wich suggest the presence of TSH monomer and Macro TSH.
Conclusion: Macro-TSH is a under recognized laboratory interference. Routine laboratory techniques described above can help diagnose this rare entity, cause of isolated TSH elevation in clinical euthyroid patients. There may be more patients with macro-TSH than expected.
The thyroid disease in pregnancy is a common clinical entity. A close dialogue between the physician and the laboratory is important in approaching such cases to allow screening for macro TSH before hormone replacement therapy is initiated for subclinical hypothyroidism.

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INTERNATIONAL NORMALIZED RATIO (INR): PERFORMANCE OF EXTERNAL QUALITY ASSESEMENT 2016 RESULTS - PNAEQ AND ECAT FOUNDATION

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Introduction: International Normalised Ratio (INR) derives from measurement of Prothrombin Time (PT) and International Sensitivity Index (ISI), and is a quantitative measure of the responsiveness of individual thromboplastin reagents to different clotting factors involved in PT measurement.
In 2014 the Portuguese National External Quality Assessment Program (PNAEQ) has established a consortium with ECAT Foundation for INR measurement.
The main objective of this study was to evaluate the performance for INR of PNAEQ participants that used two different thromboplastin reagents during 2016 and compared those with the results of ECAT participants.
Methods: The mean value and coefficient of variation (CV) of 12 human citrated plasma lyophilized samples of different INR levels ([0.98-1.08], [2.45-3.62] and [4.44-7.94]), distributed in 6 surveys during 2016, were obtained by ECAT (Algorithm A).
The analysis was focused on two lyophilized thromboplastin (human placenta and rabbit cerebral tissue).
ISI values reported by PNAEQ participants were evaluated.
All comparisons (PNAEQ versus ECAT participants and between the two thromboplastin for PNAEQ participant results) were made using a t-student test assuming normal data, since only final values were available.
Results: ISI values reported ranged from 0.91-1.13 and 1.17-1.35 for human and rabbit thromboplastin, respectively.
There were no significant differences between the PNAEQ and ECAT participant's in INR mean values for each thromboplastin (p-values > 0,05).
The mean CV by level was higher for rabbit than for human thromboplastin (table 1).
Conclusion: ISI values reported by PNAEQ participants are in agreement with WHO guidelines.
As expected PNAEQ and ECAT have no differences in terms of performance.
Although INR is meant to harmonise the PT measurement this is not completely achieved, as shown by the differences between different thromboplastins.

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Table 1: Mean CV by INR level for each thromboplastin

INR range	0.98 – 1.08 (n=2)	2.45 – 3.62 (n=6)	4.44 – 7.94 (n=4)
Mean CV - Human	5,73	9,13	8,90
Mean CV - Rabbit	6,27	9,98	10,86

Significant differences between INR values (p-value < 0.02) were observed on the 10 samples with INR > 2.45 when comparing the two thromboplastins on PNAEQ participants.

CUTANEOUS WOUND INFECTION BY NOCARDIA GRENADENSIS

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Introduction: Commonly found as saprophytes of soil and water, Nocardia species comprises a vast number of human pathogens.1 Immunocompromised individuals are particularly susceptible to infections by this agent. 1 With advances in molecular identification methods the number of recognized Nocardia species expanded.2 We report a case of a 70-year-old man presenting with cutaneous wound infection by Nocardia grenadensis.
Materials and Methods: The agent was isolated from a cutaneous swab of a sample of cutaneous wound infection of the arm of an immunocompromised farmer. Nutrient medium was used to allow agent growth. Gram and modified Ziehl-Neelsen staining were performed from positive cultures. Definitive identification of the isolate was made by 16S rRNA gene sequencing.
Results: The agent grew on sheep blood agar after 24 hours of incubation at 35 ± 2ºC in a 5% carbon dioxide atmosphere. An acid-fast, Gram positive branching rod bacteria was identified by direct examination. Automated and semi-automated equipments were not able to identify the agent. 16 rRNA gene sequencing identified the organism as Nocardia grenadensis.
Conclusions: To the best of our Knowledge, this is the second reported case of Nocardia grenadensis isolation in humans and the first one isolated from cutaneous wound infection. Molecular identification methods are an essential tool for clinical microbiology laboratory allowing accurate identification of uncommon species.

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THE IMPORTANT CONTRIBUTION OF FLOW CYTOMETRY IN THE IDENTIFICATION OF A NEOPLASTIC PLEURAL EFFUSION: FOR THE PURPOSE OF A CASE.

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Introduction: Neoplastic pleural effusion (NPE) is a frequent complication in patients with advanced stages of the disease. Early diagnosis is critical to improve the quality of life of patients. Thus, a procedure for the treatment of biological fluids has been implemented in Serviço de Patologia Clínica (SPC) of Centro Hospitalar de Leiria (CHL), combining biochemical, cytological, immunological and microbiological studies using flow cytometry (FC) to determine in a timely manner the etiology of these liquids.
Materials and Methods: A 64-year-old man followed in IPO of Coimbra with a lung adenocarcinoma in the right upper lobe of stage III A, attends the CHL Urgency Service with dyspnea. A thoracocentesis was performed and the sample was sent to the SPC. Our complete procedure was applied using FC for correct identification of the cells of this pleural fluid (PF) and a report was prepared with the diagnosis of NPE. The FC panel used includes a screening tube for lymphoproliferative syndrome with CD3, CD4, CD8, CD8, CD19, CD56 and Kappa and Lambda chains (Tube 1), and another tube with non-leukocyte cells (NLC) identification markers such as Cyto18, CD90, CD33 and CD45 (Tube 2).
Results: Hemorrhagic PF with 35.7g/L albumin, 64.8g/L of proteins, 1198 U/L of LDH, 21.2 U/L of ADA, negative microbiological and mycobacteriological examination, 2260 cells/mm3, being 15% of polymorphonuclear, 43% of mononuclear and 42% of non-leukocyte cells with a morphology suggestive of neoplastic etiology. In tube 1, no phenotypic changes were observed. In tube 2, about 60% of cells with neoplastic phenotype (cyto18 + / CD45-, CD90- and CD33-) were identified.
Conclusion: A few hours after receiving PF, with the application of our procedure of biological fluid processing, it was possible to diagnose NPE for a fast treatment and thus increasing the quality of life of the patient.

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OVERLAPPING AUTOIMMUNE DISEASES TRIGGERED BY THYMOMA- A CASE REPORT

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Introduction: Autoimmune diseases (AD) can be observed in patients with thymoma. Besides MG, thymoma is also associated with other AD, and the frequency of a second AD is about 15%. Autoimmune thyroiditis is the most common, followed by SLE and rheumatoid arthritis. The most frequent neurologic AD in thymoma patients are Isaac's syndrome (IS), acquired neuromyotonia (NM) and limbic encephalitis (LE). We report a 36 years old female with the diagnosis of thymoma B2, one year after being diagnosed with MG. She was submitted to a left anterior mediastinotomy. Two years after, CT scan showed growth of the same mass and worsening of bulbar symptoms with concomitant positive antinuclear antibodies (ANA) and antineuronal antibodies (AN-ab).
Materials and methods: Because of symptoms exacerbation, the laboratory was asked to perform AN-Ab. During indirect immunofluorescence (IF) for AN-Ab we detected strong ANA positive and used HEp-2 cells to confirm it. We used IF in different substrates: Neurologic Mosaic with nerve, cerebellum, intestine and pancreas; Encephalitis Mosaic with transfected cells for receptors NMDA, AMPA, GABAB and CASPR2 e LGI1 ; HEp-2 cells and Crithidia luciliae. Then, we performed immunoblotting with EUROLINE Paraneoplasic and ANA Profile and ds-DNA ELISA.
Results: ANA detected by (IF) were positive (1/1280) with an homogeneous pattern (AC-1), ANA Immunoblot (IB) was positive for histones, Anti-DNA by IF in a Crithidia luciliae substrate was also positive, Anti-dsDNA by ELISA was 86UI/Ml (<20UI/mL). IF in transfected cells was positive for CASPR2 and Euroline Paraneoplasic showed positive anti-CV2 +++ and anti-titin ++.
Conclusion: With thymoma diagnosis, clinicians should always include the possibility of overlapping AD associated with thymoma enlargement, possibly due to thymus escape of autoreactive T lymphocytes causing autoimmunity. Further studies are required to better understand the relationship between AD and thymoma.

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ROTATIONAL THROMBOELASTOMETRY – WHEN PLASMA-BASED COAGULATION ASSAYS DON'T HOLD THE ANSWER

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Introduction: Rotational Thromboelastometry (ROTEM) is a viscoelastic method for the whole blood evaluation of haemostasis. It allows for the continuous monitoring of the interactions between coagulation factors, inhibitors and cellular components during the formation of the blood clot and its lysis. Due to its advantages over standard plasma-based coagulation tests, mainly when applied to the haemostatic evaluation of surgical procedures with an elevated haemorrhagic risk and in traumatic coagulopathies, ROTEM has suffered an increase in utilization both as a point-of-care test and in clinical laboratories.
Case Report: A 57-year-old male, with a personal history of hepatocellular carcinoma, cirrhosis, arterial hypertension and hypercholesterolemia was admitted to the ward for an elective hepatic transplant. Laboratory evaluation during the induction phase showed: Hb 15.6 g/dL, Htc 46.2%, Plts 90.109/L, PT 15.4s, aPTT 29.3s, Fibrinogen 3.2 g/L, D-dimers 0.44 ug/mL. Intraoperatively the patient showed clinical signs of blood dyscrasia and ROTEM was performed. Forty minutes after the initiation of the ROTEM the trace revealed a pattern of decreased maximum clot firmness (MCF) compatible with a state of hyperfibrinolysis:

- EXTEM – LI30 97%, LI60 4%, ML 98%;
- INTEM – LI30 98%, LI60 6%, ML 99%;
- FIBTEM LI30 100%, LI60 17%, ML 89%;
- APTEM within normal ranges.

Once the cause of bleeding was identified, anti-fibrinolytic therapy and fibrinogen were administered with resolution of the haemorrhagic state without the need for blood transfusion.
Discussion/Conclusion: As explained by the cell-based model of haemostasis, plasma-based coagulation tests are inadequate predictors of haemorrhagic risk and suboptimal for monitoring coagulopathies or to guide transfusion therapy. When compared with ROTEM and as presented in our case, this viscoelastic method has the unique feature to make an early identification of patients with increased fibrinolysis which in turn allows for the initiation of specific anti-fibrinolytic therapy. The possibility of targeted therapy is associated with decreased transfusion requirements as well as a reduced incidence of transfusion associated adverse events and improved patient outcomes.

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INAPPROPRIATE REQUESTING OF GLYCATED HEMOGLOBIN (HbA1c)

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Background: Clinical Orientation Norm (number 033/2011) for diabetes mellitus recommends glycated hemoglobin (HbA1c) testing at least twice per year in patients with stable diabetes mellitus and quarterly in patients whose therapy has changed or who are not meeting glycemic goals. To identify the magnitude of inappropriate requesting we determined the prevalence of requesting outside guidance with HbA1c in patients with diabetes mellitus as a model.
Methods: we examined data on all HbA1c tests (Arkray Adam HA-8160 HbA1c, Menarini®) from in and outpatients between January to December of 2016. Requests were classified as "appropriate" or "too soon" (over requesting). Knowing biological half- life of HbA1c "too soon" repeat testing was defined as a request interval within 90 days. Logistic regression analysis was performed using age, sex, HbA1c and requester.
Results: There were 32908 tests (98 per day). 21908 (66.6%) were repeat samples (13945 duplicates, 5785 triplicates, 2178 quadruplicates or more). Of the repeat requests, 6.8% (1487) were "too soon" relative to guidance. The cumulative distribution of the repeat tests was 2.5% (552) within 30 days of the initial test, 2.8% (612) within 60 days and 1.5% (323) within 90 days. Tests requests "too soon" were more common in poorly controlled and older patients - ≥65 years- ($p < 0.001$). Compared with primary care, secondary care (hospital requesters) requested more tests "too soon" (57% of total).
Conclusion: Inappropriate requests represent 4.5% (1487 tests per year) of all HbA1c measurements. Poor clinician understanding of the timeframe for HbA1c change may contribute to this practice, so better education and introduction of computerized minimum retest interval guidelines should reduce such over-requesting.

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EQA QUANTIFICATION HBA1C DIABETES– LONG-TERM AND SIGMA ANALYTICAL PERFORMANCE FOR TWENTY ONE PORTUGUESE LABORATORIES

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Introduction: Glycated hemoglobin (HbA1c) is crucial to monitor and diagnose diabetes. Six sigma (σ) metrics combine bias, precision and allowable total error (Tea), and can be used for assessing the quality of the analytic phase.
The main objective was to apply a linear regression model for long-term evaluation of the precision and inaccuracy, and apply the σ metric to evaluate the laboratories performance in HbA1c quantification.
Methods: A linear regression model was applied to HbA1c results of 12 EDTA blood samples with different concentrations, to evaluate the long-term analytical performance and σ value of 21 laboratories from 2014 to 2016 that participate in PNAEQ (external quality assessment program).
The variables introduced to define the long-term performance were the LCVa and total analytical Bias and were obtained by comparing individual results with the consensus mean of each round, after outliers exclusion. The σ value was calculated using Tea based in the minimum analytical performance goals of the biological variation.
We evaluated also the number of laboratories that fulfill the minimum analytical performance goals based on the biological variation (CVa and Bias).
Results are expressed in for IFCC aligned systems (mmol/mol.

Results: The median LCVa was 2.4% [1.3-5.2]. The LCVa was less than 0.58 times the total biological variation (diagnostic testing) for 94% laboratories and was less than 0.75 times the within biological variation (monitoring testing) in 29% of the laboratories.
The median Total Bias was 2.0% [0.2-6.0]. 65% of the laboratories had a total bias less than 0.375 of the total biological variation.
The median σ value was 1.7% [0.1-4.6]. 41% of the laboratories had a σ value less than 2.0 and 59% had a σ value more than 2.0, when evaluated with an Tea of 6,7%, based on minimum performance criteria of biological variation.
Conclusion: As reflected by results the overall performance needs to be improved. Assessment of quality on the σ scale has the advantage of providing evidence of global laboratory performance taking into account random and systematic errors, and should be used for identify and prioritize improvements needed in the analytical quality of laboratory examinations.

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QUANTIFICATION OF CLOZAPINE AND NORCLOZAPINE BY LC-MS/MS IN PLASMA SAMPLES: THE IMPORTANCE OF CLZ / NCZ Ratio

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Introduction: Clozapine (CLZ), is a second generation antipsychotic used in the treatment of patients with schizophrenia refractory to standard treatment. It is metabolized by the hepatic cytochrome P450 1A2, originating norclozapine (NCZ) that is the major pharmacologic active metabolite. The metabolism and bioavailability of CLZ and NCZ depends on patient's cytochrome activity and on drug interactions. Together these variables contribute to intra and inter-individual variability in plasma CLZ concentration.
The determination of CLZ/NCZ ratio has been recommended to achieve a better therapeutic monitorization, being considered that a CLZ/NCZ ratio ≥ 2 is indicative of a metabolic saturation. This work aimed to implement the simultaneous measurement of CLZ and NCZ by liquid chromatography-tandem mass spectrometry (LC-MS/MS) allowing ratio estimation and therapeutic optimization, minimizing adverse effects.
Methods: The LC-MS/MS system used was a Waters Alliance 2695/micromass Quattromicro. The chromatographic separation was accomplished with a Mediterranea SEA 18 5 µm, 2.1 x 150 mm column. Calibrators and controls were purchased from Chromsystems. 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 327 > 270 for CLZ and 313 > 270 for NCZ. Deuterated compounds were used as internal standards: CLZ D4 (331 > 270) and NCZ D8 (321 > 192). An injection volume of 50 µL was used for a total run time of 6.5 minutes, after protein precipitation of the serum sample (100 µL).
Results: The required functional sensitivity and reproducibility for both CLZ and NCZ over a suitable dynamic range for TDM was achieved. Reproducibility was excellent for both compounds (CV < 7% for CLZ and < 8% for NCZ). Quantification limit was 2.3 ng/ml for CLZ and 10.9 ng/ml for NCZ.
Conclusion: This LC-MS/MS method proved to be specific, accurate and sensitive for quantification of CLZ and NCZ allowing the CLZ/NCZ ratio determination. It is simple, fast and appropriate to be adopted in the clinical laboratory routine for therapeutic drug monitoring, preventing adverse effects and ensuring adherence to the treatment.

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SIMULTANEOUS MEASUREMENT OF IMATINIB, NILOTINIB AND DASATINIB IN PLASMA BY TANDEM MASS LIQUID CHROMATOGRAPHY: IMPLEMENTATION OF A SIMPLE, RAPID, PRECISE AND SPECIFIC METHOD

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Introduction: Imatinib (IMAT), nilotinib (NIL) and dasatinib (DAS) are tyrosine kinase inhibitors (TKIs) with high activity to receptor and non-receptor tyrosine kinases that became first-line drugs for Bcr-Abl chronic myeloid leukemia (CML). The metabolism, bioavailability, efficacy

and adverse effects are highly dependent on intra-individual characteristics showing diverse response regarding to therapeutic success, drug resistance, dosage optimization and clinical complications. The therapeutic monitoring (TDM) of plasma levels assumes an important role in achieving an effective clinical response, promoting therapeutic adhesion and reduction of side effects. We have implemented in our laboratory a tandem mass liquid chromatography (LC-MS/MS) method that allows the simultaneous measurement of the 3 TKIs.
Methods and Materials: Chromatographic separation in the Waters Alliance 2695/micromass Quattro micro system was accomplished with a C18,5 µm 10x2.0 mm (Guard Cartridges Hypersil BDS) analytical column and a convenient gradient mobile phases system with positive electrospray ionization detection. The specific transitions m/z in the multiple reaction monitoring mode were: 494 > 394 (IMAT), 530 > 289 (NIL), 488 > 401 (DAS), 502 > 394 (IMAT-D8) e 530 > 289 (NIL-D4). Plasma calibrators and controls were purchased from Chromsystems. A sample volume of 100 µL plasma EDTA was used after convenient protein precipitation from the acetonitrile addition. Calibration concentrations ranged from 483-2400 ng/ml (IMAT), 517-1479 ng/ml (NIL) and 79.9-301.0 ng/ml (DAS).
Results
The method achieved the required functional sensitivity and reproducibility, for all TKIs over a suitable dynamic range for TDM, with an overall retention less than 4 minutes. Runtime was fixed in 6′ and injection volume in 50µL. Reproducibility was excellent for all compounds (CV < 9 %) and all calibration curves displayed excellent linearity, with r2 > 0.998.
Conclusions: This LC-MS/MS approach provides a simple, rapid, precise, and specific method for the quantification of TKIs in small amounts of plasma from CML patients, optimizing patient treatment management including clinical success and cost reduction.

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MULTIPLEX PCR IN CHILDREN'S RESPIRATORY TRACT INFECTIONS

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Introduction: Respiratory tract infections (RTI) are common in children. The identification of the pathogens is usually performed in order to implement the adequate therapeutic measures, to reduce the use of antibiotics, or to provide specific therapy to contacts.
In the last years multiplex-PCR (MPCR) systems started to be used to identify RTI pathogens. MPCR systems have a good sensitivity, require a reduced sample volume and time expense and may help in the clinical management of RTI. We intend to evaluate the results of two years of analyses of the nasopharyngeal swabs (NPS) from RTI in children up to 18 years.
Methods: NPS were tested using the FilmArray system (FilmArray® Respiratory Panel, Idaho Technology) that detects respiratory pathogens by real-time PCR, within one hour from receipt, with simultaneous detection of 21 targets: Adenovirus (ADV), Bocavirus, Coronavirus 229, HKU1, NL63, and OC43, Metapneumovirus, Influenza A, and the subtypes H1, H3, and 2009 H1, Influenza B, Parainfluenza Virus 1-4, Rhinovirus/Enterovirus (RHV/EV), Respiratory Syncytial Virus (RSV), Bordetella pertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae.
Results: 875 NPS were analyzed between November 2014 and July 2016. 68.2% of the analysis were requested by the emergency department, 4.5% by the intensive care unit, 2.4% by the hepatic transplant unit and 25% were requested by other departments. Respiratory pathogens were detected in 685 samples (78.3%). From those, 357 samples (61.5%) were from children under 12M and 198 samples (29%) were from children aged 2-5Y. RSV and RHV/EV were the virus most frequently detected in infants under 12M. In children aged 2-5Y, the most frequently detected viruses were RHV/EV, followed by ADV.
Conclusions: MPCR system was mainly used in children under 2-years–old. In the first year of life, RSV and RHV/EV were the virus most frequently detected. In older children, RHV/ EV were the most frequently detected virus. RTI may take a severe course in small children therefore, the rapid identification of the pathogens by FilmArray® Respiratory Panel may allow the implementation of the adequate therapy and avoid the unnecessary use of antibiotics.

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IMPACT OF THE LEAN PRINCIPLES ON LABORATORY WASTE REDUCTION: MAXIMIZING PRIMARY TUBES IN TOTAL AUTOMATION MODELS

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Introduction: A successful Lean system requires a comprehensive view of the entire production process and the understanding of what is waste not adding value to the product. In the clinical laboratory the impact of Lean principles sure depends on the defined organization

models pointing to different relations with the kinds of waste. Among these wastes, the total automation models (TAM) achieves reduction on waiting times, transport, movement, human repetitive actions and errors or defects. The aim of this work is to evaluate the waste reduction associated to the utilization of a sole serum primary collection tube in a consolidated TAM with chemistry, drugs, serology, hormonology, immunology, tumor and cardiac markers, compared to the present utilization of multiple tubes in our laboratory.
Material and methods: It was rated the profile of requests per patient, from the routine chemistry, in a single day (n = 642) and calculated the total primary serum tubes collected having regard to the current division into different areas in stand alone automation models. A simulated model was used to estimate the quantification of the total number of tubes reduction considering the TAM projected for our laboratory, the estimated sample volume and the optimization of work flows.
Results: A total of 1049 serum tubes collected to the 642 patients were evaluated. With the TAM projected to our lab and the consolidation of 120 serum tests in a single tube (40 for chemistry, 15 for immunology, 14 drugs, 28 for hormonology, 23 serology), one aliquot for protein electrophoresis and joining out the auto-immunity area, a total of 35,08 % tubes will be reduced. This data represents a global reduction of 79983 tubes per year.
Conclusions: The Lean principles in the implementation of a TAM laboratory corresponds to effective saving with a significant reduction of total collected tubes. Added to patient comfort the reduction in circulating tubes also simplifies operations, reduces processing time, transport, movements, stock areas, and minimizes pre-analytical errors.

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IS LANCEFIELD ANTIGEN SUFFICIENT FOR PRESUMPTIVE IDENTIFICATION OF BETA-HEMOLYTIC STREPTOCOCCI?

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Introduction: Streptococci genus harbours an important number of agents responsible for human disease. Streptococcus dysgalactiae subspecies equisimilis (SDSE) has recently emerged as significant pathogen of this genus, sharing the clinical picture with Streptococcus pyogenes.1-2This subspecies has Lancefield group C or G antigens and rarely A antigen.1 Here we present a case of prosthetic joint infection due to group A SDSE.
Materials and Methods: The agent was isolated from a sample of synovial fluid. Agent identification was performed with Vitek 2® and Walk Away® equipments. Lancefield group carbohydrate antigen was determined by agglutination. Identification of the isolate was confirmed by 16S rRNA gene sequencing.
Results: A coccus shaped positive for Gram staining bacteria was identified which presented beta-hemolysis in sheep blood agar. Agglutination was positive for Lancefield group A. Automated equipments and molecular methods identified the agent as SDSE.
Conclusions: Species assignment of beta-hemolytic streptococci based on determination of Lancefield group can lead to misclassification. Better understanding of the epidemiology of SDSE infections is possible with correct classification of this agent.

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ACUTE ERYTHROID LEUKEMIA – CASE REPORT

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Introduction: Acute Erythroid Leukemia (AEL) is a rare disease that is characterized by the myeloproliferation of erythroblastic precursors. There are two subtypes: pure erythroid leukemia, comprised almost exclusively by erythroblast, and erythroleukemia, comprised by a heterogeneous population of erythroid and myeloid precursors.

AEL can manifest in any age, although rare in children, and can be a de novo disorder or an evolution of myelodysplastic syndrome.
Material and Methods: The diagnosis of AEL was achieved using a combination of techniques in the bone marrow (BM) samples: cellular morphology, immunocytochemistry (Ki67, CD34, MPO, CD68, ALK, PS100, cytokeratins, CD20, CD23, glycophorin, TdT), immunophenotyping (CD34,CD38,CD117, CD7, CD71,CD105, CD36, CD10, CD16, CD15, CD2, HLA-DR, CD45, CD123) and cytogenetic (chromosome banding).
Case Report: 29 month-old male patient with a history of ankle fracture at the age of 15 months. Seeks medical attention in the Emergency Room of CHVNG/E presenting pain in the same ankle, causing him to wake at night, for a fortnight. Other symptoms were pallor, painless cervical and inguinal lymphadenopathies of elastic consistency, traumatic bruises in all limbs, unbalance and broad-based gait.
The lab results were: anemia (10.2g/dL), thrombocytopenia (47*109/L) and LDH 845 U/L, peripheral blood smear with mononuclear cells with fine chromatin nucleus, nucleolus, low cytoplasm and hyper basophilic. He was also submitted to X-Ray of the pelvic region and left lower limb, bone scintigraphy, serologic tests (CMV, toxoplasma, mycoplasma, borrelia, parvovirus, EBV, chlamydia and varicella), immunologic study with immunoglobulins, C3, C4, RF and ANA, all negative.
The patient was transferred to the IPO Hospital where the BM studies were non-conclusive due to a misrepresentative sample. The anemia and thrombocytopenia worsened and blasts were still present in the peripheral blood smear. In a second BM study, the sample was hypercelular, with a morphology suggestive of acute leukemia with erythroblastic differentiation.
Conclusion: AEL is an aggressive entity and a big diagnostic challenge, demanding a multidisciplinary involvement for a correct management.

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HACEK BACTERAEMIA: A VALUABLE CLUE FOR ENDOCARDITIS - CASE REPORT

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Introduction: Aggregatibacter is a gram-negative, facultative anaerobe bacilli belonging to a group of microorganisms known as HACEK (Haemophilus, Aggregatibacter, Cardiobacter, Eikenella, and Kingella). These bacteria, although classically considered causative agents of culture negative endocarditis, have been increasingly recovered thanks to modern culture systems with enriched media.
Material and methods: The diagnosis was achieved using blood cultures and biochemical tests
Case presentation: A 42-year-old male with a past medical history of Non-Hodgkin Lymphoma in complete-remission for eight years and graft-versus host disease (secondary to allogeneic bone marrow transplant) in treatment with long-term corticosteroid presented to the emergency department (ED) with fever of unknown origin.
He mentioned precordial discomfort, dysphagia with several months of evolution, mild productive cough and weakness for a week. He reported fever >39ºC and a syncope the previous day. On physical examination he was hypotensive (80/53 mmHg) and tachycardic (111 bpm). Complete blood count revealed leukocytosis (12,75 x 109/L). The other complementary diagnostic tests were normal.
A presumptive diagnosis of sepsis was established and septic screening protocol started with collection of 2 bottles of blood cultures; empiric therapy with Ceftriaxone was initiated.
After 44h incubation in BacT/ALERT® 3D system, the blood cultures were positive. Colonies grew fastidiously on chocolate agar plate over a 48-hour period. The biochemical identification (VITEK 2 systems) revealed Aggregatibacter segnis.
The patient had a favorable evolution. He was discharged and referred for Outpatient Parenteral Antimicrobial Therapy (OPAT). After microorganism identification, the assisting doctor was immediately contacted and the patient summoned back for further evaluation of a probable unrecognized endocarditis.
Conclusions: Identifying patients with endocarditis requires a high index of suspicion but when some typical agent of endocarditis appear in blood cultures, that diagnosis must be thoroughly evaluated.
This case report reinforces the importance of blood cultures collection in patients presenting with fever of unknown origin.

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MALDI-TOF IS BROKEN! AND NOW? LET'S GO BACK TO CLASSICAL MICROBIOLOGY TESTS!

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Introduction: Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) offers the possibility of accurate, rapid, inexpensive identification of bacteria, fungi, and mycobacteria isolated in clinical microbiology laboratories.(1) In our laboratory we routinely use Vitek MS (biomerieux®) for germen identification (ID) giving clinician short-time results. When MS breaks the classical microbiology tests (CMT) are absolutely needed for a faster ID orientation. We highlight the need of use rapid tests like oxidase, catalase, coagulase and solubility bile test.
Matherial and Methods: We simultaneously evaluated 79 strains using MS and CMT: 25 P. aeruginosa; 5 S. maltophilia; 28 coagulase negative Staphylococcus (CNS); 3 S. aureus; 8 Streptococcus spp.; 2 non Streptococcus spp.; 6 S. pneumoniae; 2 alpha-hemolytic (viridans) Streptococcus (VGS); obtained from urine (18), blood (26), bronchial secretions (32) and purulent exudates (3).
The strains ID on the MS were performed according to the manufacturer instructions.
As CMTs we used oxidase test; catalase test; coagulase test and solubility bile test. All the CMTs were performed according to the manufacturer instructions.
Results: The results from MS and CMT were similar for all ID except for 3 VGS that were identified as S. penumoniae by MS and were solubility bile test – negative. This misidentification is also described by other authors. (2; 3)
CONLUSIONS
The rapid tests described above are very useful not only when MS is broken but also when we have doubts on the MS ID as we do have for S. pneumoniae and VGS. For these strains, CMT allows a more accurate ID. The CMT provide a good orientation on the ID of the germen and should never be putted a side. We here emphasize the importance of CMT in microbiology.

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RETROSPECTIVE REVIEW OF ALL SAMPLE STUDIES FOR HEMOGLOBINOPATHIES SCREENING IN A CENTRAL HOSPITAL BETWEEN 2012 AND 2016

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Background: Hemoglobinopathies (HbP) are the most frequent group of genetic disorders worldwide. They have become much more common recently in northern and central Europe, including Portugal, due to immigration. They fall into two main groups: thalassemia syndromes (α- and β-thalassemia) and structural Hb variants (abnormal Hbs).[1] Diagnosis of HbP can result from either a clinical suspicion of a disorder of globin chain synthesis or from an occasionally abnormality detected during screening.[2] The World Health Organization estimates that 5,2% of the world's population carries a significant variant.[3] The aim of this study was to analyze the incidence of HbP in all samples studied for HbP screening in a central hospital during the last 5 years.
Methods: Institutional retrospective observational study performed in a central and university hospital.
A total of 2190 samples, from January 2012 to December 2016, were included in the study.
Hb A2, F and Hb variants were measured from EDTA whole blood samples on the VARIANT II® analyzer (BIO-RAD) and subsequently electrophoresed on HYDRASYS® (Sebia).
Some Hb variants were sent to an external laboratory for determination by Molecular Biology methods.
The clinical data were obtained through consultation of the individual clinical process.
Results: A total of 2190 samples were studied, of which 469 (21,4%) had genetic Hb disorders. These 469 samples corresponded to 376 patients. After eliminating the duplicates (n = 93) it was found that 62% (n = 233) of the genetic disorders present were β-thalassemia (n = 1 major and n = 232 minor) and 38% (n = 143) were structural Hb variants of which 0,27% (n = 1) Hb AE as well as Hb Himeji, Hb Setif, Hb Strasbourg, Hb Porto Alegre; 0,53% (n = 2) Hb SC as well as Hb A2 delta variant; 0,80% (n = 3) Hb AC as well as Hb Koln; 1,06% (n = 4) Hb Indianópolis; 2,66% (n = 10) Hb AD; 3,99% (n = 15) Hb SS; 5,59% (n = 21) Hb Lepore and 20,74% (n = 78) Hb AS.
Conclusion: Early detection and characterisation of the HbP is crucial.
The present study demonstrated that the most frequent HbP were β-thalassemia minor and HbAS, confirming studies already performed. High level of Hb Lepore were also observed in agreement with studies carried out in the Northern Region of Portugal.[4]
As our sampling included HbP screening samples and not the whole population, we found higher levels of genetic hemoglobin disorders.

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CATECHOLAMINE-INDUCED CARDIOMYOPATHY: A CLINICAL CASE REPORT

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Background: Cardiovascular disease comprises a large spectrum of clinical conditions ranging from unstable angina pectoris to acute ST-elevation myocardial infarction being chest pain the most usually symptom; however, it may be challenging to diagnose correctly, especially in the emergency room, due to the ambiguous way in which pain is characterized by some patients. Cardiac troponins are sensitive and specific biomarkers used in the diagnosis of myocardial infarction that are released into bloodstream when cardiac myocytes are damaged, however, since troponin elevation indicates the presence, not the mechanism, of myocardial injury, there are many clinical conditions that cause troponin elevation and, therefore, that physician should be aware of to have a clear understanding of the related pathophysiology and effectively make a differential diagnosis.
Methods: A hypertensive female patient, 64 years old, went to the emergency room due to constant retrosternal pain, which was aggravated by inspiration, starting a few hours before with dorsal irradiation, associated with dyspnea at rest, asthenia, hypersudoresis, dizziness and headache. No other significant symptoms were reported.
There were no significant alterations to the objective examination. Analytically, hs-cTnI of 19.8 ng/L evolves to 27, 5 ng/L (3h later).
After evaluation of the ECG, as the patient did not presented echocardiographic criteria suggestive of acute coronary syndrome and excluding pulmonary embolism and acute aortic syndrome the patient was admitted to the Internal Medicine service for chest pain of unclear etiology. CT Scan and urinary metanephrines were ordered for pheochromocytoma (PCC) screening.
Results: 24-hour urinary Normetanephrine and Metanephrine: 1386 µg/24h and 96 µg/24h, respectively. In the CT Scan a solid nodule with 27x22x38mm was observed depending on the right suprarenal gland. The patient was diagnosed with PCC and proposed for surgery.
Conclusions: This clinical case report describes an uncommon presentation of a PCC mimicking a cardiomyopathy and, therefore, illustrate the importance of including PCC in the differential diagnosis of patients presenting symptoms of a myocardial infarction, as early treatment may prevent serious morbidity and mortality.

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HEPATITIS B VIRUS (HBV) GENOTYPES IN CHRONIC HBV INFECTED PATIENTS STUDIED IN CENTRO HOSPITALAR LISBOA NORTE (CHLN) FROM OCTOBER 2012 TO DECEMBER 2016

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Introduction: There are eight different HBV genotypes: A, B, C, D, E, F, G and H. These genotypes exhibit different AgHBe seroconversion patterns, core and pre-core mutations, severity of hepatic disease and also therapeutic response. In Portugal, Genotypes A and D are predominant. Genotypes C, E and F were also identified in portuguese and immigrant patients.
OBJECTIVES: This study aimed to characterize HBV genotypes in chronic HBV patients studied in CHLN from October 2012 to December 2016.
Material and methods: One hundred and twenty seven whole-blood samples containing EDTA as anticoagulant were collected from chronic HBV infected patients. The viral load was determined by quantitative RT-PCR assay (VERSANT® HBV DNA 1.0 Assay, Siemens, and COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0, Roche Diagnostics). The genotype determination was performed with two different sequencing methods. From October 2012 to March 2016 the genotype determination was performed with TRUGENE® HBV Genotyping Kit, Siemens. From March to December 2016 the genotypes were determined using the Abbott® HBV Sequencing assay.
Results: A total of 127 patients were evaluated, 80 males (62.99%) and 47 females (37.0%), with a mean age of 41 years. According to the HBV genotype the distribution was: Genotype E – 54 patients (42.52%), Genotype A – 37 (29.13%), Genotype D – 29 patients (22.83%), Genotype F – 3 patients (0.02%), Genotype B – 2 patients (0.02%) and Genotype C – 2 patients (0.02%). No patients were found with G or H genotypes.
Conclusions: According to our study, which covers the period of four years and three months, Genotype E was the most prevalent, followed by Genotype A. Although Genotype E is the most prevalent in African immigrants, who represent a large percentage of Lisbon inhabitants, more data would be necessary to identify the origin of the patients included in our study.

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HEMOGLOBIN VARIANTS WITH ELETROPHORETIC MOBILITY SIMILAR TO HEMOGLOBIN S

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Abstract
Hemoglobinopathies are among the most common inherited diseases around the world and are one of the world's major health problems. They are monogenic diseases of autosomal recessive transmission resulting from mutations affecting the genes responsible for the synthesis of globin chains. Abnormal hemoglobins (Hb), named Hb variants, are caused by structural defects resulting from an altered amino acid sequence in globin chains, being Hb S the more frequent and pathogenic/disease associated.
The aim of this work was to identify and characterize Hb variants with mobility similar to Hb S when using common laboratorial methodologies, such as isoelectric focusing and high pressure ion exchange chromatography (HPLC).
Hemoglobin analysis was performed by isoelectric focusing and HPLC. Globin chain variants were classified in alpha or beta type by reversed phase high performance chromatography. Hb S was confirmed by the solubility test [1, 2]. In order to identify the rare Hb variants, molecular analyses were performed in patient's DNA.
From 2010 to 2016, in the routine practice of our laboratory, 601 cases of variants of Hb were detected with mobility Hb S-like. Amongst them, 433 were confirmed as being Hb S (72.0%). Others hemoglobins also with clinical relevance, Hb D and Hb Lepore, were prevalent, 90 (15.0%) and 61 (10.2%), respectively. The remaining 17 cases were classified as rare (2.8%) and 10 of them were identified by molecular studies as: Hb Maputo (1), Hb G-Coushata (1), Hb Summer Hill (1), Hb Setif (1), Hb G Waimanalo (1), Hb D Iran (1) Hb Oleander (1), Hb Ottawa (1), Hb Etobicoque (1) and Hb Matsue-Oki (1). Hb Matsue-Oki was found in compound heterozygosity with the –α3.7kb-thalassemia deletion.
We can conclude that combining the results obtained by the different biochemical methodologies allow the presumptive identification of the more prevalent variants, namely Hb S, Hb D and Hb Lepore, and direct the molecular study for the definitive identification. This study also revealed that several rare variants have similar mobility as Hb S and, consequently, some safety measures should be applied in order to achieve their accurate identification. A correct laboratorial diagnosis is essential for proper patient's clinical management and genetic counselling.

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BURKHOLDERIA PSEUDOMALLEI AND MELIOIDOSIS: CASE REPORT

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Introduction: Melioidosis is caused by Burkholderia pseudomallei, commonly found in soil in Southeast Asia and Northern Australia. This is a highly pathogenic bacterium, resistant to a wide range of antimicrobials, leading to case fatality rates exceeding 70% due to inadequate treatment. Worldwide, about 165,000 human melioidosis cases per year are estimated, from which 89,000 people die. The diagnosis can be difficult due to the diverse clinical manifestations and the inadequacy of conventional bacterial identification methods.
Case Report: 66 year-old man, caucasian, resident in Australia, spending holidays in Portugal, with a past clinical history of benign prostatic hyperplasia, hyperuricemia, Insulin-treated type 2 diabetes mellitus, melioidosis in 2012, who presented to the emergency department of the Abrantes Hospital, on October 19, 2016, for low back pain and fever. On examination, the patient was hemodynamically stable with only a positive Murphy's sign. Laboratory tests revealed Glucose 252 mg/dL and CRP 11.76 mg/dL, without any other relevant alterations including Urinalysis or Renal Ultrasound. The patient undergone both lumbosacral spine CT scan and MRI that revealed osteomyelitis of L5 with an abscess. Due to the persistent fever and inflammatory parameters elevation despite antibiotic therapy with Ceftriaxone, Vancomycin and Metronidazole, on November 21, he was referred to the Neurosurgery Department of Hospital de São José, Lisbon, where the abscess drainage was performed and the collected sample sent for microbiological testing. B. pseudomallei was not suspected and the cultures were negative. During hospitalization, although all blood cultures remained negative, the fever persisted and a bone marrow sample was obtained and sent to INSA, which was informed that melioidosis was a strongly suspected diagnostic hypothesis. In fact, cultures (and PCR testing) were positive for B. pseudomallei. The patient also developed inflammatory signs on the right knee from where the same microorganism was isolated.
Conclusion: This case highlights the importance of careful collection of personal history, with the inclusion of melioidosis as a diagnostic hypothesis in patients that came from endemic zones, with infectious signs that don't respond to the usual antibiotic therapy.

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DIAGNOSTIC IMPORTANCE OF MEASURING SERUM EFFUSIONS TUMOR MARKERS IN PATIENTS WITH CANCER - A CASE REPORT

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Background: Gastric cancer is the fourth most common cause of cancer-related death in the world. [1] It remains difficult to cure, as most patients present with advanced disease.
When ascites is present, the cytological examination, as well as measurement of tumour markers (TM), can predict the malignacy of a lesion visualized in radiology imaging.
Trapé J, Molina R, et al in 2012[2] validated criteria showing the utility of TM in the differential diagnosis of cancer in serous effusion (SE).
Methods: We used Trapé and Molina criteria to analise a SE of a female aged 52 which had had gastric adenocarcinoma, G2, of gastric antrum with pelvic metastasis in 2012. HER2 negative. Under palliative chemotherapy of 5th line.
On admission to the emergency service (12-05-2016) she had food intolerance for solids and liquids and increased ascites (3800 mL were drained, with no signs of peritonitis).
Results: Analytical exams in serum revealed PCR 23.90 mg/L, albumin 1.21 g/dL and total proteins 3.07 g/dL. Hepatic and renal function preserved. Anemia with hemoglobin 8.3 g/dL and leukocytes 3.18 x 103/µL with 38.4% polymorphonuclear cells (PMN) were present. No other significant changes.
Cytological examination (CE) of the peritoneal SE showed 100 Erythrocyte/µL and 100 Cells/µL (Neutrophils 5%; Lymphocytes 19%; Monocytes 6%; Macrophages 11%; Mesothelial cells 40% and 19% of not identified cells with malignant suspicious characteristics).
TM were measured in serum (CA125 195,4 U/mL, CA19.9 771,7U/mL, CEA 9,1 µg/mL) and in peritoneal fluid (CA19.9 779,5 U/mL; CEA 185,5 ng/mL), just as adenosine deaminase 1 U/L.
Conclusion: The normal cell count in the cytological examination is not always consistent with the benignity of the SE. As a rule, in our Clinical Chemistry lab, CE is performed for all SE. Otherwise the presence of cells with malignant characteristics wouldn't have been observed. Suspicion was confirmed by pathological anatomy.
Applying the criteria validated by Trapé and Molina we were abble to predict the malignancy of the fluid. According to these criteria, the relation between TM in SE and serum was useful for the diagnostic assessment in this patient: CEA (SE)/CEA (serum) >1.2 with not enough elevation of PCR, ADA or PMN. [3]

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SERUM FERRITIN EVALUATION – CLASSIFICATION OF AN ADULT POPULATION USING DIFFERENT REFERENCE VALUES

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Introduction: Serum ferritin (SFer) is the parameter that best correlates with iron stores. There are no established cut-offs because of great variability and influence by inflammation.

In our laboratory (LAB) normal values are 20-250 ng/ml in men, 10-120 ng/ml in premenopausal women (PreW) and 10-250 ng/ml in post-menopausal (PosW).
World Health Organization (WHO) considers deficiency as SFer <30 ng/ml in both genders and overload >300 ng/ml in men and PosW and >160 ng/ml in PreW. Also anemia as hemoglobin <12 g/dl in women and <13 g/dl in men.
European Association for the Study of the Liver (EASL) guidelines 2011 rates overload >300 ng/ml for men and PosW and >200 ng/ml for PreW.
Objectives: To classify patients in iron deficiency or overload according to SFer and to correlate with anemia, using different reference values: LAB, WHO and EASL.
Materials/methods: Retrospective study was performed using analytical results of SFer and complete blood count in 18650 analytical tests of 9951 patients, from July to December 2016. V Cramer coefficient (SPSS) was used for statistical analyses.
Only the first results of each patient and only the patients ≥19 years were considered. Results from oncology and obstetric areas, intensive care and emergencies were excluded.
Results: Of the 9951 patients, 5801 (58%) were women with median age of 55 years and 4150 (42%) were men with median age of 59. Median SFer was 179.9 ng/ml in men and 74.4 ng/ml in women. Anemia is present in 1555 (38%) men and 2021 (35%) women.
WHO classifies with deficiency 1354 (23,3%) women and 300 (7,2%) men, while LAB only 327 (5,6%) women and 190 (4,6%) men.
EASL and WHO classify 1260 (30,4%) men with overload and LAB 1552 (37,4%). In women, EASL defines 776 (25,5%), WHO 859 (29,1%) and LAB 1140 (39%).
In anemic women, 218 (10.8%) and 572 (28.3%) presents low SFer using LAB and WHO criteria, respectively. In anemic men, 117 (7,5%) have low SFer using LAB and 174 (11,2%) using WHO criteria.
Conclusions: LAB grades more patients with overload and less with deficiency.
There is significant correlation between SFer and anemia using WHO criteria in men ($V = ,118$; $p < 0.01$) and women ($V = ,086$; $p < 0.01$).
Using WHO classification, the prevalences are more in agreement with the bibliography.

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HPV INFECTION AMONG HIV-POSITIVE MEN: A THREE YEAR REVISED EXPERIENCE OF AN DIAGNOSIS LABORATORY

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Abstract: The spread of HIV epidemics globally has increasingly drawn attention to the interaction between HIV and the "classic" sexually transmitted infections (STIs). A consensus has grown that other STIs increase the spread of HIV, a hypothesis first suggested by Piot et al in 1984, following on from the early epidemiologic studies that explored the epidemiologic synergy between STIs and HIV.
However, the interaction of the many STIs with HIV is potentially complex, with the possibility of reciprocal influences on susceptibility, infectiousness, and the natural history of infections.
The authors present a 3 years revised casuistic as a reference laboratory center in sexually transmitted infection diseases diagnosis in HPV infected men.
Methods: Molecular methods such as Hybrid Capture 2 (hc2, Digene), Cobas 4800 HPV test (16/18-Cobas, Roche), Clart human papillomavirus 2 (Genomica) and PapilloCheck were used for HPV diagnosis.
The cytological results were registered with comprehensive classification system, multi-axial nomenclature SNOMED.
Results: In this period (Jan 2013 to Dec 2015) were analyzed 136 male samples by several HPV molecular methods and conventional cytology methods. From the 43 HPV positive cases, we obtained clinical information in 26 patients. In this group there is a higher incidence of HPV infections in men sex with men (MSM) / Bisexual group than in Heterosexual group [73.08%; 26.92% respectively] (p-value <0.01). The incidence of HPV in MSM/Bisexual group occurs at lower mean age, when compared with Heterosexual group. Vaccine was administrated in 30.77% of HPV positive group, mainly in MSM patients. The most frequent HPV types are: HPV6 (17.72%), HPV11 (12.66%), HPV51 (10.13%), HPV16 (7.59%).
Conclusion: The results obtained for the incidence of most frequent HPV genotypes in men and MSM are in agreement with several studies. Multiple infections were more frequent in Normal and LIEBG cytological specimens. On genotyping tests multiple infections decreased by the severity of the cytological interpretation), revealing that persistent and relapsing HPV infections are at higher risk for anal dysplasia development and malignant transformation. HPV infection appears to occur early in MSM.

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SEASONAL VARIATION IN SERUM POTASSIUM CONCENTRATION

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Introduction: According to some studies much of laboratory errors (32-75%) occur in the pre-analytical phase. There are several factors that can cause pseudohyperkalemia: mechanical, chemical, temperature, time, patient factors and contaminants.
The objective of this study was to evaluate the correlation between ambient temperature and serum potassium concentration in community samples.
Materials and methods: Potassium concentrations were estimated during the year of 2016 at LAC Dr. J Leitão Santos and retrospectively studied. Serum was obtained by venipuncture and collected in gel separating tubes (EUROTUBO). The number of samples analyzed was 11.950. These tests were performed routinely on Cobas c501 analyzer using indirect ion selective potentiometry.
The internal quality control for potassium estimation was within specifications and was consistent throughout the study period (CV < 1,5%). The performance in the national external quality assurance program was also within specifications (TE 3,99% < admissible TE 5,8%).
Temperature data were obtained from the monthly reports from IPMA.
The monthly mean potassium values and the correspondent average temperatures were plotted against the correspondent month of the year.
Results: The calculated mean value of potassium concentration is 4,62 mEq/L (RV: 3,50 – 5,10 mEq/L). Mean serum potassium concentrations rise as the temperature falls in winter (4.74 mEq/L), and the inverse occurs during the warmer summer months (4,47 mEq/L).
Discussion: Our results demonstrate an inverse correlation between ambient temperature and serum potassium concentrations. These rises with the decreasing in temperature in winter and the opposite occurs during summer months.
Because potassium leaks from blood cells into the serum/plasma at lower temperatures, the lower potassium results obtained on days when the ambient temperature is high are probably closer to the true potassium concentrations.
Laboratories should review their pre-analytical procedures where transport temperatures and rapid centrifugation after collection are main factors that help minimize these seasonal preanalytical variations, some of which, in borderline values can have significant clinical impact.

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HbA1c DETERMINATION IN A PATIENT WITH HOMOZIGOUTY FOR HAEMOGLOBIN S (HBSS)

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Introduction: Diabetes is a metabolic disease with increasing prevalence in both developing and developed countries. The evaluation of blood glycated hemoglobin A (HbA1c) is used to monitor the average blood glucose levels during the previous two to three months, which is the predicted half-life of red blood cells (RBCs). If the RBCs lifespan is decreased because of Hb abnormalities, the HbA1c level will be lower. Therefore, HbA1c determination in patients with Hb abnormalities such as sickle cell disease (homo or heterozygous state) must be interpreted with caution.
We present a case report of HbA1c determination in a patient diagnosed with homozygous (SS) sickle cell disease.
Material and Methods: HbA1c determination was performed in EDTA anticoagulated blood samples using a HPLC system (VariantTM II Turbo, Bio-Rad). Glucose was determined in the Vitros 5600 analyzer and hemogram was evaluated in the Cell-Dyn Sapphire according to manufacturer instructions. The analysis of plasma fructosamine (FA) was performed using an enzymatic immunoassay.
Results
Hemogram, glucose and HbA1c were requested to a diabetic, 38-year-old male patient. The hemogram revealed the existence of a normochromic normocytic anemia. Fasting glucose was 4.3 mmol/L (77 mg/dl). The chromatogram revealed the absence of the integration peak at the retention time characteristic of HbA1c. These findings were integrated with clinical information, which revealed that the patient was homozygous (SS) for sickle cell disease.
Conclusion: HbA1c is not adequate to monitor the average blood glucose levels in homozygous sickle cell patients. Serum FA is formed by nonenzymatic glycosylation of serum proteins, predominantly albumin and measures short term control of blood glucose for the past 1-3 weeks. Since the FA concentration of well-controlled diabetic patients may overlap with those of people who are not diabetic, the FA test is not used as a screening test for diabetes. However, when determination of HbA1c may be unreliable, the determination of frutosamine can be an alternative. This case-report emphasize how important is the integration of clinical and laboratory information.

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AUTOMATIC VALIDATION IN THE LABORATORY: A MODEL APPLIED TO URGENT REQUESTS

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Introduction: In the Clinical Pathology Service of a central university hospital, where 39% of the results are carried out urgently and Biochemistry represents 80% of this work, we have created automatic validation conditions, based on reference values, critical values and variation from previous results.
Methods: From the most frequent chemical tests, 35 parameters were chosen, corresponding to 80% of the total urgent requests and automatic validation conditions were created. The software used in the Service of Clinical Pathology (Clinidata) has subroutines that verify the conditions of validation every 5 minutes. The tests with results that fulfill the validation conditions are validated and become available to the Clinicians.
Results: This process has been in operation for about 3 months and has been gradually implemented allowing the adjustment of the validation conditions and the inclusion of a larger number of parameters. At present, it is possible to automatically validate from 55 to 65% of biochemical urgent requests, depending on the period of the day.
Conclusions: In the laboratory, this automated process represents an advantage in obtaining the results, in the optimization of the validation and subsequent availability to the clinician. Although this process is still in the initial phase, its preliminary results enable us to broaden the spectrum of the involved parameters and improve their turnaround times.

HEMAPHOGOCYTIC SYNDROME SECUNDARY TO ADULT - ONSET STILL'S DISEASE - CASE REPORT

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Introduction: Hemophagocytic syndrome (HPS) is a rare, life-threatening complication of systemic inflammatory disorders, characterized by increased proliferation and activation of macrophages with hemophagocytosis throughout the reticuloendothelial system. Adult-onset Still disease (AOSD) is one of the systemic autoimmune diseases associated with hemophagocytic syndrome characterized by prolonged spiking fever, salmon-colored rash, arthralgias, leukocytosis and other manifestations involving multiple organs. The authors present a clinical case of a hemophagocytic syndrome secundary to adult-onset Still's disease.
Case report: A 25-year-old female was admitted to our hospital with fever, anorexia, weight loss, asthenia, evanescent salmon-pink skin rash and vomiting since the week before. She had a past medical history of refractory AOSD treated with prednisolone 20 mg, omeprazol 20 mg, anakinra 100 mg and calcium carbonate 500 mg/ cholecalciferol 400UI. On admission, she had a skin rash on the trunk, face, hands and forearms. Laboratory investigations revealed normocytic anemia, leucocytosis with neutrophil predominance, hyperferritinemia, elevated erythrocyte sedimentation rate, C-reactive protein, liver enzymes, hypertriglyceridemia and hemophagocytosis visualized in myelogram. The rheumatoid factor, anti-nuclear antibody, and anti-dsDNA were negative. Blood and urine cultures were also negative. Histopathological examination of the skin biopsy revealed features of a neutrophilic dermatosis. Chest radiography and abdominal ultrasound were normal. She was treated with pulse therapy with methylprednisolone (1000 mg/ 5 days), cyclosporine 5-6 mg/Kg and anakinra (400 mg id) with clinical and analytical improvement.
Conclusion: HPS is an uncommon complication of AOSD and it shares many clinical features with underlying AOSD. Thus, the recognition of incipient HPS in AOSD patients requires a high index of suspicion. The laboratory results are crucial for the diagnosis, since it requires the exclusion of infectious, malignant and other autoimmune diseases.

25-HYDROXYVITAMIN D MEASUREMENTS AT CENTRO HOSPITALAR SÃO JOÃO: A CROSS-SECTIONAL ANALYSIS

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Introduction: In the last decade, 25-hydroxyvitamin D [25(OH)D] has generated interest among researchers and clinicians and, despite its deficiency being increasingly recognized around the world, few studies portray the Portuguese reality. Therefore, in this study we analyze 25(OH)D measurements in Centro Hospitalar São João (CHSJ, Porto, Portugal) according to age, gender, requesting specialties and month of sample collection.
Material and Methods: A cross-sectional study of 25(OH)D measurements was performed in CHSJ between January, 2012 and December, 2016. 25(OH)D level from patient serum was determined by electrochemiluminescence immunoassay. 25(OH)D status was classified as deficiency (<20 ng/mL), insufficiency (20-30 ng/mL) or sufficiency (> 30 ng/mL).

Results: A total of 79 600 25(OH)D assays were performed with a peak in 2016 (23 490) corresponding to an increase of 129% compared to 2012. The medical specialties that most contributed to the increase were rheumatology with 18 072 assays and endocrinology with 17 426. A single assay was performed in 58.3% and 51.9% of rheumatology and endocrinology patients, respectively. Most of the assays were from females (75.4% in rheumatology and 67.1% in endocrinology) aged 40-79 years (78% in rheumatology and 74% in endocrinology). In rheumatology, 35.5% corresponded to deficiency, 32.6% insufficiency and 31.9% sufficiency levels and in endocrinology, 42.7%, 31.1% and 26.2%, respectively. No significant difference was found on male/female distribution according to 25(OH)D status. 25(OH)D absolute level varied with patient's age (with levels higher in 60-79 year old patients vs 19-59 year old patients, $p < 0.001$). There was a seasonal variation on 25(OH)D levels ($p < 0.001$), with the highest levels occurring in August to October, despite medians remaining below 30 ng/mL.
Conclusion: From 2012 to 2016 the number of 25(OH)D assays more than doubled, mainly due from rheumatology and endocrinology clinics. Most were below 30 ng/mL and seasonal variation was evident with peak median levels in September. To better understand the cost-effectiveness of these measurements, evolution of 25(OH)D levels on each patient after insufficiency/deficiency assessment needs to be evaluated, as well as the consequence of intervening on 25(OH)D status on clinical outcome.

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HYPERINFECTION BY STRONGYLOIDES STERCORALIS

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Background: Strongyloidiosis is endemic in tropical and subtropical areas. Gastrointestinal symptoms such as abdominal pain, bloating or bleeding may occur. Hyperinfection can occur whenever the number of worms increases tremendously in the setting of immunosuppression, especially in patients receiving corticosteroids and HIV, transplanted or malnourished patients. We report two fatal cases of severe strongylodiosis in immunocompromised patients.
Methods: The first was a 66-years-old male with a clinical history of ANCA-P3 positive vasculitis treatment with cyclophosfamide and prednisolone. Patient presented dyspnea, hypoxemia, and purpuric skin lesions. Chest X-ray showed a faint bilateral interstitial infiltrate. Bronchial secretions and gastric aspirate were submitted for bacterial and Mycobacterium detection. Forty years prior of this episode the patient had resided for 2 years in Guinea-Bissau. The other case was a 88-years-old male with a prostate cancer and metastatic adenocarcinoma of colon 6 years ago. The patient presented a week-long history of abdominal distension, moderate abdominal pain, asthenia and anorexia associated with ascitis. Stool and ascitic fluid were examined for bacterial and parasitic detection. The patient had resided in Angola until the age of 40.
Results: In the first patient, Gram and Kinyoun staining of bronquial secretions and gastric aspirated revealed a large number of larvae typical of S. stercoralis. Microscopic examination of stool showed larvae and eggs. In contrast microscopic stool examination of the second patient showed numerous larvae but no eggs; bronchial secretion and ascitic fluid were negative for parasites. Both patients were administered ivermectin and albendazole but they died after 10 and 6 days of hospitalization, respectively.
Conclusions: Patients under immunossupressive therapy and risk factors should be regularly checked for S. stercoralis. Clinicians should be aware of this complex clinical picture and avoid a late diagnosis usually with a very poor outcome.

HEMOGLOBINOPATHIES: A FIVE YEAR REVISED EXPERIENCE OF CENTRO MEDICINA LABORATORIAL GERMANO DE SOUSA

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Abstract: The hemoglobinopathies are defined as hereditary conditions characterized by mutations in the human globin genes, leading to quantitative modifications to globin synthesis or the production of structurally abnormal hemoglobin molecules. They are the most prevalent genetic diseases in the human species and represent a serious public health problem in some parts of the world.
Distribution in the population is very variable with high prevalence in the Mediterranean countries. In Portugal, the distribution is heterogeneous, with higher prevalence in the center and in the south.
The most severe forms of hemoglobinopathies in Portugal are sickle cell disease and β-thalassemia major and intermedia [1].

Methods: We evaluated all hemoglobin electrophoresis (capillary electrophoresis) requested for hemoglobinopathies screening/diagnosis between January 2011 and December 2016. The solubility test was used to confirm HbS. All variant hemoglobins, with the exception of HbS, were confirmed by alternative methods in the Reference Laboratory (INSA).
Results: Of the 19086 patients in which the hemoglobin electrophoresis study was performed the mean age of population without pathology was 34.89 years, and the mean age of the population with pathology 35.44 (p-value 0.305).
The prevalence of hemoglobinopathies in the studied population was 9.41%, representing a prevalence of 0.02% in the national population. The highest prevalence of pathology was found in females (63.79%) (p-value < 0.001).
The main hemoglobinopathies found in the studied population (98%) were heterozygous β-thalassemia (883; 49.11%), hemoglobinopathy AS (733; 40.77%), hemoglobinopathy SS (70; 3.89%) and hemoglobinopathy AC (49; 2.73%). Other variant hemoglobins were also identified: Hb AD (27), Hb Lepore (15), Hb SC (4), Hb N-Baltimore (4), Hb Porto Alegre (4), Hb CC (1), Hb AE (1), Hb K-Woolwich (1) and Hb Setif (1).
The national geographic distribution was heterogeneous, with higher prevalence in center (12.57%), and north (10.62%). In the Lisbon great metropolitan area and south region we observed similar hemoglobinopathies prevalences (9.41% and 9.20%, respectively).
Conclusion: The main hemoglobinopathies found are in agreement with described in the bibliography referring to the Mediterranean population.
The authors emphasize the importance of screening programs for laboratory hemoglobinopathies identification and genetic counseling as a key role in improving prevention of new occurrences and timely diagnosis.

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CASE REPORT: B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA WITH MONONUCLEOSIS

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The infection by Epstein-Barr virus (EBV) is often associated with structural changes in B-lymphocytes. It is also implicated in the development of a wide range of B-cell lymphoproliferative disorders.
We describe the case of a 4-year-old female child, without previous relevant medical history, who goes to the emergency department (ED) presenting symptoms of respiratory infection – cough, sometimes inducing vomit, with white mucous nasal secretions – twice in a month, treated with antibiotics and completely resolving the symptoms.
One week after her second time in the ED, the child is analytically anaemic, with leukopenia and inversion of the leukocytic formula, as well as some reactive lymphocytes in the blood smear. The serologic study shows acute infection by parvovirus. It is assumed to be a case of bone marrow failure by parvovirus infection and the child is admitted for treatment and surveillance.
A week later, the hematologic alterations continue to worsen and the liver enzymes suddenly rise. The polymerase chain reaction (PCR) test for parvovirus is negative.
At this point, the blood smear shows 11% of mononuclear cells with immature characteristics and the child is transferred to the city's Cancer Hospital – Instituto Português de Oncologia do Porto –, where the bone marrow (BM) study reveals hipocellularity with 36% of blastic cells. Immunophenotyping suggests B-cell Acute Lymphoblastic Leukemia (B-ALL) with hyperdiploid characteristics, later confirmed by cytogenetics examination. In addition, the virology study now suggests acute infection by EBV.
Therefore, the correct diagnosis would be B-ALL with hyperdiploidy and co-infection by EBV. The patient is then submitted to the appropriate treatment scheme with good prognosis.
The morphological changes of the B-lymphocytes into reactive lymphocytes caused by EBV may have been a factor in the difficulty of the initial observation of the blood smear due to the similarities between these cells and the blastic ones. It is essential to know how to differentiate these two types of cells in order to correctly refer the patient in the ED.

A CASE OF PLASMODIUM MALARIAE WITH NEGATIVE IMMUNOCROMATOGRAPHIC TEST RESULTS

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Introduction: Immunochromatographic "rapid" tests are used worldwide for the diagnosis of malaria infection alongside microscopy. However, their sensitivity varies greatly, at least, according to species.

Objective: To highlight microscopy as mandatory and gold standard for diagnosis and species id. Alert to low negative predictive value of "rapid" tests.
Clinical case: Male, Caucasian age 36, no relevant personal history, admitted in the ER, at the 7th day of symptoms consisting of night fever (40-42ºC), with afebrile intervals of 24h, associated myalgia, right lumbar pain and occasional coughing. Resident in Angola for 8 months, having arrived in Portugal 3 days prior to admission. He neglected antimalarial prophylaxis.
Physical examination: eupneic, tympanic temp. 39,3ºC, tachycardic, normotensive, heart sounds, PA, abdominal and neuro exam reported as normal.
Chest X-ray unremarkable. Analytically: mild transaminase elevation, CRP 3,6 mg/dL, relative neutrophilia, absent leucocytosis, no changes in red cell indices (Hb 13.8 g/dL) nor thrombocytopenia (168^109/L). Negative malaria immunochromatographic test. On Peripheral blood smear (PBS) examination: very rare P. malariae schizonts (<0,0001% of erythrocytes). Left shift, hemozoin pigment and activated lymphocytes observed.
Treated with quinine 600 mg PO 8/8 h and doxycycline 100 mg PO 12/12h for 7 days. Medical discharge as asymptomatic, at the 2nd day of admission.
Discussion: PBS examination should not be considered negative until 20-40 min, 200 HPF or the whole blood film has been examined. In our case report, a short hand approach would lead to a false negative result.
The Core™ Malaria Pan/Pv/Pf test was negative in two samples. According to the manufacturer the test has 100% specificity for four species, not disclosing the sensitivity for P. malariae and P. ovale, which may be as low as 50%. Hence the false negative result in the case of an overtly symptomatic patient.
Conclusion: Rapid test results for Plasmodium spp. should be interpreted with caution, in clinical and epidemiological context. PBS examination is the international gold standard and reference method for diagnosis and species id., also allowing detection of indirect haematological signs of malaria. However, it requires a minimum time/fields examined by qualified personnel.

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DALTEPARIN INDUCED THROMBOCYTOPENIA AND STROKE – A CASE REPORT

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Introduction: Heparin induced thrombocytopenia (HIT) is an immunologically mediated adverse drug reaction to unfractionated heparin, or less commonly, to low molecular weight heparin (LMWH), in this particular case dalteparin, with potentially devastating consequences. This complication occurs as a result of antibodies binding to the complex of heparin and platelet factor 4 (PF4). These antibodies subsequently activate platelets through their Fc receptors, causing the release of prothrombotic platelet-derived microparticles, which in turn promote thrombin generation and contribute to a hypercoagulable state. The authors present a clinica case of dalterapin induced trombocytopenia and stroke.
Case Report: A 45-year-old female was admitted to the hospital with an isquemic stroke of the right anterior median cerebral arterial territory. The patient had a recent exposure to dalteparin after a right leg varicose vein surgery and a past medical history of dyslipidemia. After surgery the patient had leg pain and edema and was treated with Dalteparin until the stroke event. There was no history of thrombocytopenia pre-surgically (250000 platelets), but the first blood count at admission revealed thrombocytopenia (91000) and HIT was suspected. The immunoglobulin G anti-bodies against the PF4 were positive (26.6 U/ml) and dalteparin was suspended. The patient was treated initially with fondaparinux and after that with vitamin K antagonists. Before discharge the platelet values were within normal range.
Conclusion: This is a rare case of LMWH (dalteparin) induced thrombocytopenia in which a thrombus induced by the hypercoagulable state associated to this pathology is the most likely explanation of the stroke's etiology. There is also the hypothesis of an eventual paradoxal embolism, even without deep vein thrombosis on duplex ultrasound.

STRONGYLOIDES STERCORALIS COLITIS IN IMMUNOCOMPROMISED PATIENT

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Introduction: The infection by the intestinal nematode Strongyloides stercoralis is mainly acquired in tropical and subtropical regions. Its life cycle involves penetration of the host's skin, reaching the bloodstream and entering the alveolar space causing pneumonitis. It is then

coughed and swallowed, becoming adult in the gastrointestinal tract, where it can initiate an autoinfection cycle that allows the host to remain infected for decades.

Case Presentation: We report the case of a 39-year-old brazilian female who presented to our Emergency Department with abdominal and thoracic pain, diarrhea and nausea over a four day period. She had recently travelled to Brazil.

The patient was HIV-1 positive and had not begun antiretroviral therapy yet. Two years earlier she was presumably dIagnosed with pulmonary tuberculosis and treated accordingly.

She presented with fever, but no respiratory distress, pulmonary auscultation was normal and there were no signs of peritoneal irritation. Laboratory results revealed hypochromic, microcytic anemia and elevated C-reactive protein, without eosinophilia. Her chest x-ray had no acute abnormalities and her abdominal ultrasound revealed thickening of the colon wall, suggestive of colitis, and nodular hepatic lesions suggestive of Candida infection. She was admitted to the hospital and treated with metronidazole and ceftriaxone. The initial workup focused in the search of causes of colitis other than parasitic. Afterwards, a microscopic stool examination (MSE) for parasitic ova and larvae was undertaken, which revealed the presence of rhabditiform larvae of Strongyloides stercoralis. The patient was treated with albendazole and discharged. Days later she was readmitted because of recurrence of the initial complaints – with persistence of Strongyloides stercoralis on MSE – and treated with ivermectin.

Conclusion: The MSE for parasitic ova and larvae was key for this diagnosis of strongyloidiasis.

It stresses the importance of taking into account not only the patient's immunity status but also travels to exotic destinations that should prompt other recommended laboratory tests like the MSE.

Equally important is the communication between Clinicians and the Laboratory for the exchange of relevant information and differential diagnosis discussion.

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ONYCHOMYCOSIS CAUSED BY NON-DERMATOPHYTE MOLD – CASE REPORT

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Introduction: Onychomycosis is a common disease that results in almost 60% of nails abnormalities.1 It is a nail infection caused by dermatophytes, yeast or non-dermatophytes molds.2 Non-dermatophyte mold infections affect mostly toenails and the most frequent agents are Scopulariopsis spp., Fusarium spp., Acremonium spp., Aspergillus spp. and Scytalidium spp.1,3

Diagnosis is made when clinical and laboratorial criteria are present; the latter including direct microscopy and fungal culture.4

The treatment of choice of onychomycosis caused by non-dermatophytes molds is itraconazole.2

Materials and methods: A diabetic 58-year-old man was admitted in dermatology outpatient clinic. On physical examination, he presented a longitudinal leukonychia in his left halux, suggestive of onychomycosis. The surface of the nail was scrapped with a scalpel blade after nail's decontamination with 70% alcohol. It was performed a direct exam with KOH (20%) and a cultural exam using Sabouraud and Mycobiotic agars.

Results: In direct bright field microscopy hyaline septate hyphae were observed.

Fungal culture revealed a pure culture of a filamentous fungi in Mycobiotic and Sabouraud agars. The surface of the colonies in Mycobiot agar was pale-rose and "cotton-like" and in Sabouraud agar was yellowish. The reverse of the colonies was pale yellow on both agars.

In the microscopic examination of the colonies with lactophenol blue, we observed septate hyphae, with erect and unbranched phialides, which formed directly from the fine hyphae. Conidia were disposed in clusters at the tip of phialides, disrupted from them and were oblong in form. This features were consistent with Acremonium spp.

The patient started treatment with itroconazole, which was effective.

Conclusions: The difficulty in evaluating the role of non-dermatophyte mold fungi cultured from nails arises from the fact that the same fungi that can be specimen or laboratory contaminants, are also occasionally found to be pathogens.5

Non-dermatophyte mold fungi are probably contaminants unless microscopy and culture correlates and the same organism is repeatedly isolated.5

Although only one sample was obtained, the presence of a pure culture and the positive response to treatment suggests that Acremonium spp. was the agent of infection.

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MEASURING VITAMIN E:CHOLESTEROL RATIO - ONE YEAR AFTER THE IMPLEMENTATION OF A LC-MS/MS METHOD AT OUR LABORATORY

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Introduction: Vitamin E (VitE) is a fat soluble vitamin that acts as antioxidant to protect the integrity of unsaturated lipids in biomembranes. Without consensus, several studies recommend that VitE concentration should be calculated as a ratio to total cholesterol. One year after the implementation of a liquid chromatography-mass spectrometry (LC-MS/MS) method we evaluated the utility of the VitE:cholesterol ratio (E/C).
Material and Methods: Considering all determinations of VitE and cholesterol in the same sample, during 2016, a retrospective study was performed. VitE was determined by LC-MS/MS according to our protocol. Cholesterol was performed in the Olympus AU5800 analyzer according to manufacturer instructions. The reference intervals were: <1Y: 3.5-8.0 mg/L; 1-12Y: 5.00-9.00 mg/L and >12Y: 5.00-20.00 mg/L, for VitE and: <18Y: 3.68-7.48 mg/g; >18Y: 4.74 - 6.50 mg/g for the E/C.
Results: In the patients <1Y, the mean VitE concentration and E/C was 10.7 ± 4.1mg/L and 8.1 ± 3.1mg/mg respectively. Of the 45 samples, 3 (6.5%) had a low VitE concentration, and 5 (10.8%) had a low E/C.
In the patients between 1-12Y the mean VitE concentration and E/C was respectively 8.9 ± 3.7mg/L and 6.32 ± 2.6 mg/mg. Of the 111 samples, 16 (14.4%) had a low VitE concentration, and 11 (9.9%) had a low E/C. Only 7 of the patients that had a low VitE also had a low ratio.
In the patients >12Y the mean VitE concentration and E/C was 10.8 ± 4.0mg/L and 6.2 ± 2.0mg/mg respectively. Of the 228 samples, 9 (3.9%) had a low VitE concentration, and 29 (12.7%) had a low E/C. Only 1 of the 29 patients was under 18Y. The mean cholesterol of the patients that had a low E/C was significantly higher than that of the patients with a decreased VitE concentration.
Conclusion: For patients under 18Y measurement of VitE concentration alone seems to be adequate to identify a VitE deficit, agreeing in most cases with E/C. For patients above 18Y VitE concentration and the E/C are in disagreement, especially in older patients which may reflect the increase in cholesterol associated with aging and the need to integrate VitE concentration with clinical evaluation. In the current modes E/C apparently does not seem to be useful.

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PERSISTENT TROPONIN I ELEVATION: A LABORATORY CHALLENGE

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Introduction: Serum troponin measurement is an important criteria for the definition of acute myocardial infarction (AMI). Results must be thoroughly interpreted, in order to detect a rise and/or fall pattern of the measurements in subsequent samples in the appropriate clinical context.
Case presentation: A 72-year-old female patient with known history of cardiac disease was admitted to our hospital after loss of *consciousness*. *Serial* measurements of serum Troponin I were persistently high (around 30ng/mL) when using the TNI STAT immunoassay in Cobas®6000 (Roche). CK, CK-MB and Myoglobin results were within the reference interval. Serial electrocardiogram showed no signs of ischemia. During hospitalization the patient never complained of chest pain.
Facing these conflicting outcomes, Troponin I was measured using assays from other manufacturers which led to results fully below the established cut off. At this point it was hypothesized that the initial troponin result was a false positive, arising from a sample interference, and the patient was discharged.
Complementary studies were conducted in order to determine the interference nature.
Methods: Rheumatoid Factor was measured in the patient serum. Polyethylene glycol (PEG) precipitation test was performed. The serum sample was fractionated by size exclusion chromatography (SEC) and troponin was measured in all serum fractions using TNI STAT assay. Studies also included a competition assay using aliquots spiked with different antibodies.

Results: Rheumatoid Factor was below cut off. PEG precipitation test showed extremely low Troponin I recovery (0,3%). Data analysis from SEC showed that there was no Troponin I in the sample and that the interfering molecule is likely to be an IgM.
Competition assay showed that the presumed IgM molecule had the ability to bind specifically to the antibody used in the TNI STAT assay.
Conclusion: The patient's troponin concentration obtained using the TNI STAT assay is a false positive result. Complementary studies results are consistent with the presence of a *Human Antimouse Antibody* (HAMA).
Despite the fundamental role of cardiac biomarkers in AMI investigation, the measurement result must be interpreted in the context of the patient clinical condition. In case of conflicting outcomes the rare event of a HAMA presence must be considered.
Acknowledgments: We thank Roche Diagnostics for performing size exclusion chromatography and the competition assay.

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COMPARATIVE ANALYSIS BETWEEN THE OBSERVATION OF URINARY SEDIMENT USING LIGHT AND DIGITAL MICROSCOPY

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Introduction: The examination of urinary sediment (US) is an integrated part of urinalysis and is an irreplaceable tool for the diagnosis and monitoring of kidney and urinary tract diseases. The reference method for the analysis of US is microscopy which can be performed in optical microscope or in automatic analyzers (AA) - digital microscopy. However, in AA, the speed and time of centrifugation of the samples and the amount of urine remaining in the tube for resuspension are not standardized which may limit the correct identification of the different elements and their accurate quantification. We carried out a study that aimed to evaluate the influence of the sample centrifugation rate to the automatic analysis of US.
Material and methods: We analyzed the US from 10 randomly selected samples using the SediMax analyzer (Menarini®) and an optical microscope (Nikon®). Each sample was first analyzed on SediMax according to the procedures recommended by the manufacturer. The analyzer homogenizes the sample and releases it in a small slide that is centrifuge and photographed. The obtained images are processed by the analyzer's software that performs an automatic identification and count of particles.
Upon the automatic analysis, 10 ml urine was centrifuged at 520g for 10 minutes, and 9 ml of the supernatant was discarded. After homogenization this volume was placed on AA using pediatric tubes and analyzed as previously. At the end of the automatic analysis, 50 µl of the sample were placed on a slide and observed in the optical microscope, magnification 40 x. Images from the 10 urine samples were analyzed according the three methods were compared.
Results: In the initial sample, the results for erythrocytes, leukocytes, epithelial cells and other structures were similar to the results of microscopic examination ($r > 0.97$ for all parameters). In the centrifuged sample, the results from AA and from microscopic examination were inconsistent ($r > 0.90$ for erythrocytes, leucocytes and epithelial cells, but $r < 0.80$ for the remain particles observed).
Conclusions: Apparently, the centrifugation step does not improve results. The analysis performed by AA is in accordance with the analysis performed by optical microscope.

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RELEVANCE OF THE INTEGRATION OF CLINICAL AND LABORATORY DATA TO THE DIAGNOSIS

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Introduction: One of the most frequently requested laboratory tests upon a suspicion of a monoclonal gammopathy (MG) is serum protein electrophoresis (SPE). Several MGs do not present a peak or present a doubtful monoclonal peak which identification is operator dependent. The reduced sensitivity of SPE may compromise the diagnosis of MGs such as MG of undetermined significance (MGUS).
Material and methods: SPE, serum and urine immunofixation (SIF and UIF) were performed using Sebia electrophoresis systems according to the manufacturer instructions. Phenotypic quantification and characterization of plasma cells in bone marrow aspirate was performed in a FACS CANTO II Flow Cytometer with the monoclonal antibodies CD138/CD38/CD19/CD56/CD45/Ig Kappa/Ig Lambda
Results: A 50-year-old male followed since 2010 at the nephrology department due to chronic renal failure, secondary to renal polycystic disease was referred in January 2016 to hematology due to a normochromic normocytic anemia. A SPE was performed and a doubtful monoclonal peak in the beta-gamma transition was detected. With the exception of anaemia, and of a reduced creatinine clearance (30 ml/min/1.73m2,stage III), no other analytical alterations were detected.
Patient was re-evaluated 3 months later. The SPE was repeated and an even more doubtful monoclonal peak, in the same transition was detected. The performance of a SIF allowed the identification of a MG IgA kappa. The immunoglobulins and the light chains concentration and the ratio kappa/lambda were in the normal range. A UIF was performed but no alterations were detected. The patient recovered from anemia, but the creatinine clearance became reduced to 25 ml/min/1.73m2. The phenotypic study of bone marrow revealed the presence of 0.3% plasma cells, of which 48% presented a phenotype different from the normal with clonality to kappa light chain.
Conclusion: Since the sensitivity of SPE is reduced, some MGUS present a SPE pattern without alterations. In this situation it is crucial to integrate results from clinical history, physical exam and laboratory analyses such as biochemistry, hemogram, SIF, immunoglobulins and light chains quantification and plasma cells phenotypical evaluation.

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THE IMPORTANCE OF THE LABORATORY IN THE DIAGNOSIS OF A CELIAC DISEASE CASE

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Introduction: We present a case of a 67-year-old female patient diagnosed with celiac disease by the Immunology Laboratory.
Case presentation: A 67-year-old obese patient attended a hepatology consultation since 2007 for the follow-up of a probable non-alcoholic fatty liver disease (NAFLD).
In November 2012, an analytical control detected an elevation of transaminases (AST: 137 U/L; ALT: 254 U/L; ALP: 197 U/L GGT: 152 U/L) with weak positive antinuclear antibody (ANA) and negative anti-mitochondrial antibodies (AMA). The patient started medication for primary biliary cirrhosis.
Anti-reticulin antibodies were detected on liver, kidney and stomach tissue (IIF) in the analytical control in July 2016. To confirm a celiac disease, the pathologist added anti-transglutaminase IgA and anti-gliadin deaminated IgG, that were positive (tTG IgA: 6265 UA/ mL; AdGA IgG: 33,4 UA/mL) and anti–endomysial (EMA) also positive. In august the patient undergoes a duodenal biopsy confirming the diagnosis of stage 3 - 3b celiac disease in the Marsh classification.
The patient started the gluten-free diet and is expected to perform further analytical checks to see if there is any improvement.
Conclusion: Celiac disease can be presented at any age with wide range of clinical manifestations or be asymptomatic.
A laboratory finding of anti-reticulin antibodies was useful to diagnose celiac disease in this patient, when there was no clinical suspicion.

CONGENITAL TOXOPLASMOSIS: A CASE REPORT

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Introduction: Toxoplasmosis is caused by infection with intracellular parasite Toxoplasma gondii. Congenital toxoplasmosis is an infection of newborns that results from the transplacental passage of parasites. These infants may be asymptomatic at birth, but some of them may manifest a wide range of signs and symptoms like chorioretinitis, epilepsy and psychomotor retardation. Acute infection in women during pregnancy is generally asymptomatic and most cases are diagnosed with perinatal serological screening. The severity of symptoms in newborns depends on the timing of diagnosis and initiation of treatment.

Materials and Methods: A 31 years old woman was diagnosed with toxoplasmosis during pregnancy. Seroconversion occurred between the 2nd and 3rd trimester and subsequent amniocentesis was positive. She started treatment with Spiramycin, Pyrimethamine and Sulfadiazine at the 34th week of gestation. She gave birth, at the 39th week, a baby with an 9/10 APGAR and no clinical evidence of disease. Serology tests were made by automated bioMérieux immunoassay, VIDAS®.
Results: The serology at birth was IgG >200 IU/ml with negative IgM and IgA, and PCR test in blood and CSF were negative. Two months later, serology showed decrease in IgG (26.5 IU/mL) with IgM and IgA positivity. A week later, IgG increased to 1045 IU/mL, and some weeks later IgM and IgA became negative. Therapeutic protocol was started with Spiramycin, Pyrimethamine and Sulfadiazine. The PCR tests were always negative.
The child never presented evidence of disease and had a normal psychomotor development.
Conclusion: At birth, serological tests are poor diagnostic tool, since IgG can be transmitted from the mother and IgM may be negative. Furthermore, negative IgM and IgA do not exclude congenital infection, since they can be produced only months later.
Serologic screening and early treatment during pregnancy appear to be associated with reduction of frequence and severity of sequels in the newborns.

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ANTINUCLEAR ANTIBODY PATTERNS AND RELATED AUTOANTIBODIES - CLINICAL ASSOCIATION

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Background: Detection of antinuclear antibodies (ANA) by immunoassay is an important tool in the diagnosis and follow-up of patients with autoimmune diseases (AID). In general, the first test to be requested is ANA detection by indirect immunofluorescence (IFI) using HEp2, due to its great sensibility. Autoantibodies identification is further evaluated by more specific tests such as ELISA, RIA or EIT. In this study we determine the rate of ANA positivity and patterns, investigate the relationship between ANA positivity and specific autoantibodies, and its relation to autoimmune diseases.
Methods: ANA tests results of 1720 patients admitted during 2016 were evaluated retrospectively. ANA test (Hep2, EuroiMMUN®) was performed using a dilution of 1:160 in IFI test. The presence of specific auto antibodies was then evaluated by Elia (Imunocap 250®)
Results: 473 (27%) patients were ANA positive. ANA positive rate was significantly higher in female (75%) patients (p«0.001). The mean age was 57, 1. The most frequent ANA patterns were coarse nuclear speckled (49%), fine nuclear speckled (42%) and homogeneous (5%). Other patterns were found in less than 3 % of positive samples. In 75 (15,8%) ANA positive specimens, autoantibodies resulted positive. The most prevalent autoantibodies were SSA (35%), anti-ds DNA (31%) and SSB (9%). ENAs positive rate was significantly higher in female (86%) (p«0.001). The mean age was 48,9. In this group, 18 patients did not have diagnostic criteria for autoimmune disease. 57 were related to diseases such as Systemic Lupus Erythematosus (41 %) and Primary Sjogren Syndrome (12%) The most frequent clinical findings were joint pain, anemia and thrombocytopenia.
Conclusions: ANA positivity is more common in female patients. The more prevalent autoantibodies were SSA, followed by anti-ds DNA. The interpretation of these tests requires knowledge of clinical classification criteria of AID in order to contribute to appropriate diagnosis.

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SYSTEMIC LUPUS ERYTHEMATOSUS AND AUTO IMMUNE HEPATITIS: A CASE REPORT

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Abstract: Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder involving various organs such as kidneys, skin and the central nervous system. Liver involvement is normally not part of the spectrum of SLE, but is seen in up to 60% of SLE patients. Hepatotoxic drugs, coincident viral hepatitis and non-alcoholic fatty liver disease (often induced by steroids) are the most commonly described causes of elevated liver enzymes in SLE.

Liver diseases that accompany Systemic Lupus Erythematosus (SLE) disease activity generally have good prognosis and do not progress to cirrhosis.
It is important to distinguish SLE-associated hepatitis (SLE hepatitis) from auto immune hepatitis (AIH) since complications and therapies are different in the two conditions. SLE may result in end stage renal disease while AIH may lead to end stage liver disease.
Methods / results: The author's present a case report of a female patient with 30 years old, diagnosed for LES in 2005 in immunosuppressive therapy for almost 10 years with Methotrexate and Cyclosporine, with recent aggravating complaints for knee and elbow arthralgia with morning rigidity over 2 hours, abdominal pain with episodic fever and fatigue. In recent laboratory tests evaluation, beside the homogeneous ANA pattern with rising titers and specific antibodies for LES, we found a citoplasmatic filamentous pattern suggestive for ASMA, found to be F-actine antibodies confirmed in VSM 47 transfected cells, with borderline liver function tests.
Discussion: With the occurrence of changing patterns on ANA, is compulsory that the laboratory investigation is orientated towards specific markers for AIH, which usually do not occur in SLE, like soluble liver antigen (SLA), Liver-pancreas, smooth-muscle antibody (ASMA) with specificity for F-actin and microsomal autoantigens, such as anti-liver kidney antibodies (anti-LKM antibody).[1,3,4]
Conclusion: Differential diagnosis of elevated liver enzymes in patients with SLE as either non-specific hepatic involvement or as AIH is demanding. Histology may be essential to distinguish AIH in SLE from non-specific hepatic involvement in SLE.
To distinguish whether the patient has primary liver disease with associated autoimmune clinical and laboratory features resembling SLE - such as AIH - or the elevation of liver enzymes is a manifestation of SLE remains a difficult challenge for the treating physician.

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HEMOLYSIS RATE IN AN EMERGENCY DEPARTMENT – 7 YEAR MONITORING EXPERIENCE

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Background: Serum hemolysis is the most prevalent pre-analytical error and an important laboratory quality indicator. It might interfere in the determination of the concentration of several biochemical tests. It generates a significant number of rejected samples and the need for blood specimen re-collection. This results in the prolonged laboratory response time and might cause a delay in patient care. It's also a waste of human, pharmaceutical and clinical resources.
Methods: The monthly hemolysed sample rate of Unidade Padre Américo – Centro Hospitalar do Tâmega e Sousa, Portugal, emergency department was calculated since May 2009. The data includes total hemolysis rate and hemolysis rate that required a blood sample re-collection.
Results: During this period, two major events made a big impact on the data. In May 2011 transition of visual detection to the automatic semi-quantification of hemolysis index increased the total hemolysis rate, while in January 2015 changes on hemolysis definition criteria (following recommendations of IFCC-WG-LEPS, January 31, 2014 version) decreased it.
The lowest rate of sample re-collection due to hemolysis in the emergency department was 4.1% obtained in March 2012.
Conclusion: Because of the nature of the emergency department the hemolysis rate was always higher when compared to other hospital departments (inpatients and outpatients) and the performance benchmark of 2% was not reached.

HARMONIZING RESULTS OF THE 3 CLINICAL CHEMISTRY LABORATORIES OF PATHOLOGY SERVICE OF CENTRO HOSPITALAR DE LEIRIA EPE

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Introduction: Centro Hospitalar de Leiria (CHL) is accredited by Joint Comission International (JCI). JCI's standards (1) states that all patients must have the same health quality services across the 3 Hospitals of CHL: Hospitals of Alcobaça, Leiria and Pombal (3H). CHL's Pathology Service (PS) has 3 laboratories units with 4 clinical chemistry analizers (4L), 2 Beckman Coulter Aus 480 and 2 Aus 2700, responding to urgency, routine and inpatients of the 3H. Some of the CHL inpatients also moves across the 3H, when specialized health services are needed. When monitored with the data obtained from any of the 4L, it is as if obtained from only one clinical chemistry analyzer.

Material and Methods: The reference change value (RCV = 2,33 X √(CVa2 + CVi2) indicates whether the difference between two consecutive determinations for a single patient is pathophysiologically relevant, therefore we can use it to evaluate the risk of clinical decision between different analyzers.
PS defines the same allowable measurement uncertainty (CVa), on the 4L assays, with the same quality control charts for all the 4L, using the same lots of BioRad's Multiqual Assayed controls, using a 2sd Westgard as Rule-Out.
Using the intra-individual biological variation (CVi) for all the 4L assays, for serum and urine, we can calculate the relevant pathophysiologically detection capacity (RCV) with the defined CVa on the 4L.
When temporary unsolved bias (surpassing the CVa) appears between the 4L, after confirmation with BioRad Laboratory Peers (BLP) and patients distribution data's charts on Modulab Gold (PDD), we apply curve correction factors to maintain all 4L assays harmonized, until the final correction or elimination of the bias.
Results: The PDD, shows the patients results distribution for each assay on each analyzer. This is our most powerful tool to detect a bias that affects the patients, or shows us that the problem is only on control samples or that the correction was efficient to maintain the harmonized response of the 4L.
The BLP is our main tool to confirm a shift on control samples stability, and defines whether our control targets should be adjusted for all 4L.
Conclusion: All 4L of the 3L, are harmonized with the same RCV for the monitoring of all the 3H patients.

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