Congress Abstracts

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Scientific Committee

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| Cristina Jorge

Cristina Maria Rego de Freitas Mendes Jorge obtained her graduation in Medicine at Lisbon University on July 31, 1990. She is a Graduate Assistant in Nephrology and has been performing her duties at Centro Hospitalar de Lisboa Ocidental / Hospital de Santa Cruz mainly in the area of kidney transplantation, having collaborated in scientific meetings, publications and presentation of works in this area of Nephrology. She has been a member of the Board of the Portuguese Transplantation Society since November 2009.

| Immunologic monitoring KTx

Although kidney transplantation constitutes the best treatment option for those with chronic kidney disease, the renal graft is subject to several threats and pathologies which can compromise its viability. These aggressions can be categorised as immunologic (like T cell or antibody mediated rejection) or nonimmunologic (hypertension, diabetes, or toxicity from immunosuppression). The medical follow-up of kidney transplant recipients has not changed much in the last decades – kidney function is evaluated through biochemical parameters (creatinine serum levels, calculated GFR, presence or absence of proteinuria), or even by histologic analysis of a kidney biopsy (considered the gold standard for a precise diagnosis). But all these methods have limitations and different aggressions may have the same phenotype. Furthermore, when serum creatinine rises, there has already been a significant loss of renal function. Therefore, there is urgent need for new methods that can precociously and accurately detect the cause of a kidney graft lesion before it becomes irreversible.

In this presentation, the author summarises some of the new and/or most promising methods for an accurate diagnosis in kidney transplantation.

These include the analysis of gene expression in the peripheral blood, the evaluation of cell free DNA from donor origin, or tests based on extracellular vesicles, which can be analysed either in blood or urine.

The author hopes the liquid biopsy and precision medicine will be a part of our daily routine in following kidney transplant recipients in the near future and believes these new methods will help us all reach better outcomes for our patients.

| Sara Querido

Sara Querido Conde, born on January 29, 1983, in Minde.

Graduated in Medicine by *Faculdade de Medicina* de Lisboa in 2008. Residence in Nephrology *(Internato Complementar)* at *Centro Hospitalar do Médio Tejo* between April 2011 and October 2016.

Since March 2017, Assistant Nephrologist at *Hospital de Santa Cruz, Centro Hospitalar de Lisboa Ocidental*. Main activity at the Renal Transplantation Unit António Pina.

Author and co-author of several national and international oral communications and posters, as well as several publications, including first authorship in 14 scientific articles in peer reviewed international journals.

PhD student at Nova Medical School, developing a doctoral project in the area of viral infection and kidney transplantation.

|The role of viral markers in monitoring the immune status of kidney transplant patients

Kidney transplantation (KT) is the best treatment for eligible patients with end-stage kidney disease. Contemporary immunosuppression for KT reduced the incidence of graft rejection but increased the risk of infection and virally mediated malignancies. Until recently, no reliable biomarker has emerged to guide clinicians in adjusting the level of immunosuppression. However, some recent publications identified innovative viral biomarkers as promising elements to assess global functional immune competence, to predict post-transplant immune-related adverse events and, eventually, to customize immunosuppression.

A promising strategy is the monitoring of peripheral blood levels of torquetenovirus (TTV). Although TTV can be detected in up to 90% of healthy individuals and it has not been associated with any specific disease, peripheral blood levels of this virus might reflect the overall strength of innate and specific immunity. Few publications evaluated whether quantification of TTV could be a predictive biomarker for infectious risk in solid organ transplant patients. Studies demonstrated that low TTV levels were associated with graft rejection and higher TTV levels correlated with a higher risk of infection. However, the ideal threshold for reduction of immunosuppression is yet to be determined, as well as the best time points to measure TTV viremia.

JC virus (JCV) is a polyomavirus whose primary infection occurs often during childhood. Following infection, it become latent, but persist in the urinary tract. The incidence of JCV reactivation after KT is undefined. The few published data evaluating JCV viruria after KT, revealed a clinical favorable outcome of JCV viruric KT patients. JCV viruria may be associated with adequate immunosuppression, potentially leading to a lower acute rejection rate. Thus, monitoring JCV viral load might also provide a way to adjust immunosuppression.

| Luís Ramalhete

Luis Ramalhete, master in Biomedical Engineering, Biomedical Scientists, with more than 26 years' experience in immunogenetics, Immunology and transplantation. Currently the supervisor of Laboratório de Alosensibilização e Serologia HLA, CSTL-T, IPST, IP - Instituto Português do Sangue e da Transplantação. Involved in the Portuguese Kidney exchange program and in several transplantation investigation studies.

Anti-HLA antibodies utility in allograft allocation and in post- transplantation monitoring

The presence in the recipient of preformed antibodies to HLAantigens (anti-HLAab) directed against donor (DSA) and is impact in the outcome of transplant is well described and is strongly associated with increased risks of rejection and allograft loss. To determine the presence of these anti-HLAab several techniques can be employed in the laboratory setting, traditionally these anti-HLAAb, were mainly identified by complement-dependent cytotoxicity (CDC); however, the introduction of new technologies such as multiplex bead array immunoassays Luminex (Lx) has provided alternative methods. The sensitivity and specificity provided by these Lx assay, combine with several in-house modifications (e.g. EDTA addition to remove complement interference or C1q biding anti-HLAab), have provided the tools to the precisely identify anti-HLAab in the case of highly immunized patients (Cleary identifying the window of permissible antigens to safely transplant), or providing the means of transplant risk stratification. In the post-transplant evaluation systematic monitoring of anti-HLAab DSAs allows for the early diagnosis of ABMR disease and subsequent specific treatment and adjustment of immunosuppressive therapy.

| Mariana Monteiro

Instituto Ciências Biomédicas Abel Salazar (ICBAS), University of Porto (UPorto).

Mariana P. Monteiro completed medical degree at Faculty of Medicine at University of Porto (UPorto), the Endocrinology Specialist Training at Hospital de Santo António, Porto, and pursued her postgraduate PhD studies at Institute of Biomedical Sciences Abel Salazar (ICBAS, UPorto) and at Imperial College London. Currently, is Associate Professor at ICBAS, General Coordinator of the Unit for Multidisciplinary Research in Biomedicine (UMIB) and Principal Investigator of an interdisciplinary research on Endocrinology and Metabolism, with a particular focus on obesity, diabetes and bariatric surgery, with over 100 peer-reviewed indexed publications.

| Medical evaluation before and after obesity surgery

Obesity is a complex and multisystem disease, which together with its related comorbidities, threatens to jeopardize the health life expectancy gains achieved over the past century. For severe obesity, bariatric surgery is currently the single most effective treatment in achieving sustained long-term weight loss.

Targeted medical-assessment of patients with obesity prior to intervention and follow-up over the years after bariatric surgery is essential for achieving and maximizing the success of weight loss and obesity-related diseases management, as well as for long-term patient well-being. By prompting reorganization of gastrointestinal tract anatomy, bariatric surgery can result in nutrient malabsorption and trigger significant micronutrient deficiencies. Ensuring patient nutritional education before surgical interventions, lifelong vitamin supplementation and timely and targeted biochemical profiling is essential not only for ensuring patient safety but also to optimize surgical outcomes.

Raising physicians' awareness over the need to appreciate the interplay between negative energy balance and the risk of common nutrient deficiencies, as well as a good communication between clinicians and the laboratory are crucial to achieve these goals.

| Ana Paula Rodrigues

Ana Paula Rodrigues is a Public Health Doctor since 2010 working on infectious disease surveillance at the Epidemiology Department of the National Institute of Health Doutor Ricardo Jorge since 2013. She coordinates *Rede Médicos-Sentinela* (a research network of family doctors) and the Portuguese National Serological Survey to Coronavirus Disease-19.

She has a Master in Research Methodologies in Health (Universidad Autónoma de Barcelona).

|Portuguese National Serological Survey to Coronavirus Disease-19: results from the second phase

Ana Paula Rodrigues[1] on behalf of the ISN COVID-19 team
[1] Epidemiology Department, National Institute of Health Doutor Ricardo Jorge

Introduction: Seroepidemiological studies allow estimating more precise cumulative incidence when compared with results obtained from the SARS-CoV-2 RNA detection test. In this context, the first Portuguese COVID-19 National Serological Survey (ISN COVID-19) had as primary objectives to: i) monitor changes in SARS-CoV-2 seroprevalence along time in order to characterize the extent of SARS-CoV-2 infection and its immunity within the Portuguese population; ii) determine seroprevalence in specific age groups and Health Regions; and ii) determine the proportion of seropositive cases after vaccination.

Methods: ISN-COVID-19 was an observational, cross-sectional study. The first phase was realized between May-June 2020 and the second phase in February-March 2021 (after the 3rd COVID-19 epidemic wave). A non-probabilistic sample of 8,463 people residing in Portugal, aged between 1 and 79 years old, was selected among users of clinical laboratories or hospitals (total of 352 collection points). Sociodemographic, epidemiological and clinical data were collected using a questionnaire and a blood sample was collected from each participant. Qualitative detection of SARS-CoV-2 specific IgM (anti-Spike protein) and IgG (anti-Nucleocapside protein) were performed using chemiluminescent microparticle assay (CMIA) for all participants. For those who had a positive result of IgM (anti-S) or IgG (anti-NP) and for all vaccinated participants IgG (anti-S) was also measured.

Results: National seroprevalence was 15.5 % (14.6 - 16.5 %), being 13.5 % (12.6 – 14.4 %) attributed to previous infection. These values were higher than the accumulated incidence of SARS-CoV-2 infection reported by the National Surveillance System (7.9%). The lowest seroprevalence was estimated in Algarve (7.7 %), Madeira (6.2 %) and Azores (5.8 %) and among people aged between 70 to 79 years (8.9 %).

The concentration of IgG (anti-S) was higher among those who had 2 doses of COVID-19 vaccine compared with those who had 1 vaccine dose or who got a previous infection. Among people who didn´t report being vaccinated before the study enrolment, IgG (anti-S) concentration was higher if a symptomatic infection was reported and if it occurred 31 to 90 days before the study enrolment.

Conclusions: The estimated SARS-CoV-2 seroprevalence was accordingly the very intense COVID-19 epidemic observed in Portugal between October and February. As our results suggest an antibody waning after 90 days, seroprevalence might underestimated the real COVID-19 attack rate. Nevertheless, our estimates were higher than the COVID-19 incidence rate

reported by the national surveillance system. Considering the elevated proportion of seropositive participants after 2 COVID-19 vaccine doses it is plausible that SARS-CoV-2 seroprevalence at population level will increased in the forthcoming months.

| Hélder Mota Filipe

- Degree in Pharmaceutical Sciences (University of Lisbon). and PhD in Pharmacology (University of Lisbon).
- Associate Professor of Pharmacology and Clinical Pharmacy (Faculty of Pharmacy, University of Lisbon)
- Principal investigator (ISBE- evidence-based health institute, University of Lisbon)
- Executive Member of the National Ethics Committee for Clinical Research
- President of Portuguese-speaking Countries Pharmacists Association
- Former Vice-President and President of Infarmed.
- Former member of the Management Board, European Medicines Agency (EMA)

|COVID-19 vaccines

There are four main platforms for vaccine development. All of them have been used to produce vaccines against Covid-19. In Europe, 4 vaccines are currently approved, and others are under evaluation by the European Medicines Agency (EMA). The rapid development of vaccines resulted from the combination of a set of unique conditions. Despite the common indication, vaccines have different characteristics that affect the way they can be stored and distributed, requiring a complex organization from a logistical point of view. Due to the short time that has passed since the beginning of the pandemic, important questions are still impossible to answer either about the disease, or about the efficacy and safety profile of vaccines. The quantity of vaccines available and inequity of access at the global level are also a major concern. This presentation intends to address, through a critical view, the themes identified above.

Oral Communications and Posters

|CO1

REFERENCE VALUES OF HAEMATOLOGICAL RATIOS IN A HEALTHY ADULT POPULATION: THE EPITEEN COHORT STUDY

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Introduction: Inflammation is both a perpetuating pathologic process and a biomarker in a myriad of diseases. The relationship between inflammation and blood counts is well-established, with increasing evidence supporting the clinical utility of a new set of biomarkers comprised of ratios between blood cell populations. Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), Systemic Immune-Inflammation Index (SII), Lymphocyte-to-Monocyte Ratio (LMR) and Haemoglobin-to-Platelet Ratio (HPR) show promise as biomarkers of progression and outcomes in several diseases, including carotid stenosis, atrial fibrillation, and pulmonary carcinoma. However, there is a lack of studies regarding reference values for set ratios, particularly in healthy adults.

Objective: This work aims to propose a reference range for NLR, PLR, SII, LMR and HPR for a European healthy adult population.

Methods: Data from the EPITEEN population-based birth cohort was analysed, including adults born in 1990. Participants were evaluated at 26 years of age, with a total of 1211 healthy adults. Reference intervals were calculated non-parametrically, using the 2,5 and 97,5 percentiles of the distribution for each ratio. Differences between genders were evaluated by a t-test (normal distribution assumed). P<0.05 was considered statistically significant.

Results: Participants had a mean age of 26.82 ± 0.50 years (mean ± sd), with 48.9% (N=551) of males. Values for the blood counts were in the normal range – Hb 14.12 ± 1.36 g/dL, leukocyte count 14.12 ± 1.36 x 103/µL and platelet count 235.50 ± 55.05 x 103/µL. The ratios had different reference intervals according to gender, apart from NLR - 1.71 ± 0.83 (female) vs 1.70 ± 0.88 (male), p=0.771; PLR 118.75 ± 34.55 vs 106.84 ± 33.03, p < 0.001; SII 421.42 ± 204.59 vs 370.67 ± 186.16, p < 0.001; LMR 4.79 ± 1.49 vs 4.05 ± 1.23; p < 0.001; HPR 0.06 ± 0.01 vs 0.07 ± 0.01, p < 0.001.

Conclusions: This study is, to the best of our knowledge, the first to establish reference intervals in a healthy adult population, with a significant sample size, for haematological ratios. Participants were followed since birth and recruited from different hospitals. Reference values differ according to gender in most indices. In the future, we intend to evaluate reference ranges for different age categories.

|CO2

NEW FORMULA FOR β-THALASSEMIA SCREENING

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Introduction: β-thalassemia (BT) is one of the most common genetic diseases in the world. BT diagnosis is based on multi-step algorithms including expensive tests available in specialized laboratories, thus rapid, widely available and low-cost screening tests are highly sought after. Recently, based on the study of 293 samples (ADVIA Hematology System, Siemens, USA) from Italian children a discrimination index (DI) for BTT screening (DI-BTT) was developed, calculable using the following formula: (RBC × MCHC × 50/MCV)/CHr, where: RBC - red blood cells, MCHC - mean cellular hemoglobin concentration, MCV - mean cellular volume and CHr - mean reticulocyte hemoglobin. This index was significantly higher in BTT patients clearly separated them from normal controls and patients with iron deficiency anemia (IDA).

Objective: We aimed to evaluate the discriminating capacity of the new formula (RBC × MCHC × 50/MCV)/CHr for BTT screening in adults using Sysmex XN10 (Kobe, Japan) parameters.

Materials and methods: This study is a retrospective analysis of samples from consecutive adult outpatients. Selection criteria for the group I (BTT carriers) were: HbA2 > 3.5% and MCV < 80fL. IDA patients (group II) were defined according to Hb < 11g/dL, MCV < 80fL, with previous history of normal Hb and MCV. Normal controls´ (group III) selection criteria were: 12g/dL < Hb < 16g/dL, 80fL < MCV < 100fL. RBC, MCHC, MCV and reticulocyte hemoglobin equivalent (Ret-He) measured on Sysmex XN10 (Kobe, Japan) were used to calculate DI-BTT = (RBC × MCHC × 50/MCV)/Ret-He. Results were expressed as means ±SD and compared between the groups by use of a Student´s unpaired t-test, considering statistically significant p-values of <0.05.

Results and discussion: Group I was comprised of 14 samples with MCV 63.02±0.91fL, Group II – 17 patients with MCV 65.24±2.84fL, Group III – 58 subjects with 88.70±4.32fL, all the groups matched by age and gender. The mean DI-BTT value in Group I was higher than in Group II, and notably higher than in Group III: 7.19±0.91 vs. 5.68±1.06 vs. 2.76±0.44 (p < 0.001).

Conclusion: Our results support that DI-BTT allows BTT to be distinguished from normal controls and IDA patients, even in cases with low MCV (61-69fL). The national and international population will benefit from further confirmation of the new formula´s screening value.

|CO3

THE PROSTATE HEALTH INDEX (PHI) DENSITY IN PROSTATE CANCER DETECTION: DOES IT OUTPERFORM PHI OR THE PROSTATE-SPECIFIC ANTIGEN (PSA) DENSITY?

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Objective: To evaluate the diagnostic performance of the PHI density (PHID) and compare it with the performance of the PHI alone and of the PSA density (PSAD).

Materials and Methods: 232 men with no previous history of prostate cancer (PCa), scheduled for a prostate biopsy, were enrolled based on a PSA level between 2 and 10 ng/mL. PSA, free PSA (fPSA) and [-2]proPSA (Hybritech calibration), were measured on the Beckman Coulter Access 2 analyzer. PHI was calculated as ([-2]proPSA/fPSA)$\times\sqrt{}$PSA and the total prostate volume (TPV) was measured on transrectal prostate ultrasound, or on multiparametric prostate magnetic resonance imaging. PHID was estimated as PHI/[TPV in mL] and PSAD as PSA/[TPV in mL]. The outcomes were PCa or clinically significant PCa (csPCa) on biopsy, defined according to the Prostate Cancer Research International Active Surveillance (PRIAS) study criteria. Parametric and non-parametric tests, ROC curves and logistic regression analysis were performed. Diagnostic sensitivities, specificities and predictive values were calculated, considering both outcomes.

Results: On univariate analysis, PHI, PSAD and PHID were predictors of the outcomes ($p<0.001$). For PCa, the area under the ROC curve (AUC) was higher for PHID (0.823) than for PHI (0.779), PSAD (0.776) and PSA (0.609). For csPCa, the AUC was also higher for PHID (0.851), but closer to the AUC of PSAD (0.819) and PHI (0.813). On multivariate analysis, both PSAD and PHID offered a gain of 7% in predictive accuracy for PCa or csPCa when added to the base model (PSA and PHI). For equal sensitivities (90%) for PCa, PHID and PSAD offered the highest specificities (37%), allowing to spare the same percentage of biopsies (22%), and missing the same number of cancers (n=11). For csPCa, PHI and PHID had similar specificities (35.8% and 39.6%), sparing approximately the same number of biopsies (25%-26.3%) and missing almost the same number of csPCa cases (8-10). PSAD reached the highest specificity for csPCa (50.0%), allowing to spare more biopsies (32.8%) and maintaining the same csPCa detection rate (9 missed cases).

Conclusions: Within the PSA range of 2-10 ng/mL, PHID has a better diagnostic performance than PHI, for overall PCa, but very close to the PSAD performance. For csPCa, PHI and PHID perform almost equally, but PSAD shows a superior diagnostic performance.

|CO4

IMPORTANCE OF THE PERIPHERAL SMEAR IN THE CORRECT EVALUATION OF BASOPHILIA – ALDER–REILLY INCLUSIONS IN MAROTEAUX–LEMY SYNDROME

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Introduction: Maroteaux–Lemy syndrome or mucopolysaccharidosis (MPS) type VI is an autosomal recessive lysosomal storage disease that causes mutations of the ARSB gene that in turn leads to ASB enzyme deficiency and thus accumulation of

dermatan sulphate in the lysosome. This defective metabolism leads to musculoskeletal abnormalities, visual and hearing impairment, respiratory failure, heart disease and ultimately, death. The disease might be rapidly progressive (early onset, severe symptoms, death at an early age) or slowly progressive (later onset, milder symptoms). There are over a hundred genetic mutations. Diagnosis is based on clinical symptoms, urinary glycosaminoglycan excretion, measurement of ASB enzyme activity, and mutational analysis of the ARSB gene. The cornerstone of treatment is enzyme replacement therapy (ERT). Early detection is of utmost importance, as the institution of ERT can prevent irreversible organ damage.

Case Description: A 32-year-old female patient with a history of Maroteaux-Lemy syndrome was evaluated as part of a routine follow-up. Diagnosis was established in 2004 by mutation analysis, describing a de novo mutation of p.L72R (c.215>G), followed by continuous ERT therapy. Over the years, the patient suffered musculoskeletal deformities causing severe functional limitation, seizures, cardiac and respiratory abnormalities, corneal clouding and optic nerve atrophy. Clinical parameters were normal except for relative basophilia of 3.5% (Sysmex® XE-2100D haematology analyser). A blood smear was prepared and analysed (Sysmex® SP-1000i and CellaVision® DM96) and revealed several cells of the granulocyte lineage that contained azurophil inclusions. By further analysis it was possible to differentiate two very similar looking but distinct populations: normal appearing basophil granulocytes and neutrophils with Alder–Reilly inclusions that are pathognomonic of MPS.

Conclusions: Early diagnosis of MPS and initiation of ERT are extremely important to avoid permanent organ damage. Early in the course of the disease, especially in case of the slowly progressive form, clinical clues might be scarce. Evaluation of the peripheral blood smear and correct assessment of basophilia might provide an important clue that prompts further investigation of this rare disease.

|CO5

COLD AGGLUTININS IN THE CONTEXT OF COVID-19

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Introduction: Cold agglutinins are autoantibodies that recognize erythrocyte antigens at less-than-physiological temperatures, leading to their agglutination and extravascular hemolysis through complement fixation, resulting in anemia, typically without hemoglobinuria. Cold agglutinins may be seen with the primary/idiopathic cold agglutinin disease or secondary cold agglutinin syndrome (SCAS). The antibodies are typically IgM. SCAS can be triggered by viral infections, such as Mycoplasma pneumoniae infection, Epstein–Barr virus, influenza, autoimmune disorders, or lymphoid malignancy.

Aim: Here, we present a study of presence of cold agglutinins identified in the context of Coronavirus disease 2019 (COVID-19), in the span of one year in our Hospital.

Methods: In this retrospective study we analyzed all the blood samples where the presence of cold agglutinins was identified, performed in our laboratory between March 2020, the beginning of the COVID-19 pandemic in Portugal, and March 2021. Among those patients, we selected the ones where SARS-CoV-2 RT-PCR test was positive.

Results: In the span of one year we identified a total of 35 patients with presence of cold agglutinins. Nine of these patients were identified in the context of SARS-CoV-2 infection. All of these COVID-19 patients presented with increased LDH. Of these, 3 patients presented with hemolytic, macrocytic anemia, of which 2 required blood transfusion. These patients had low haptoglobin levels and a positive Coombs test. Mycoplasma pneumoniae and EBV serologies were performed in these patients and were found to be negative. Among the 26 patients with cold agglutinins and negative SARS-CoV-2 test the majority were found in the context of monoclonal gammopathy or autoimmune disorders.

Conclusions: Infection by SARS-CoV-2 could be a trigger for transient crioagglutinin development, however more studies are necessary to establish a clear relationship. Despite minimal in-vivo hemolysis found in most patients, these antibodies are of clinical significance given their implications for laboratory assessment, requiring pre-heating of the samples before analysis, and potentially for renal replacement therapy, requiring warming of dialysis circuit, relevant considering the multi-organ dysfunction observed in severe COVID-19.

|CO6

COMPARISON OF ADAMTS13 ACTIVITY MEASUREMENT BY ELISA AND A NEW CHEMILUMINESCENCE ASSAY

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Introduction: the guidelines from International Society on Thrombosis and Haemostasis (ISTH) for the diagnosis of Thrombotic Thrombocytopenic Purpura (TTP) recommend that ADAMTS13 activity results should ideally be available in 72h. An early result allows for the management of the condition with the monoclonal antibodies caplacizumab and rituximab. Currently, ADAMTS13 activity is measured by TECHNOZYM® ADAMTS-13 Activity ELISA in our centre, a labour-intensive manual method. HemosIL AcuStar ADAMTS13 Activity, a new quimioluminescent immunoassay, may help decrease the turnaround time for ADAMTS13 activity measurement.

Aim: to evaluate the performance of the new automated HemosIL AcuStar ADAMTS13 Activity assay with the TECHNOZYM® ADAMTS-13 Activity ELISA assay.

Patients and Methods: 40 citrated plasma samples from patients with suspected or confirmed TTP were analysed. 27 retrospectively from frozen samples and 13 newly arrived samples. TECHNOZYM® ADAMTS13 Activity ELISA assay was performed manually and read by spectrophotometry on Milenia Kinetic Analyzer Microplate Reader and HemosIL AcuStar ADAMTS13 Activity assay was performed on BIO-FLASH®. The assays were compared by Bland-Altman Plot and Passing-Bablok Regression in R with “BlandAltmanLeh” and “mcr” packages.

Results: ELISA assay classified 36 samples as negative (activity > 20%), 3 samples as positive (activity < 10%) and 1 sample as borderline (activity between 10% and 20%). There was a 100% agreement for positive samples and 97,2% for negative samples. The borderline sample was considered positive by the HemosIL AcuStar ADAMTS13 Activity assay.

The bias was 0,013 and the upper a lower limits of agreement were -0,386 and 0,413 respectively. The Pearson’s r was 0,864 and the regression equation y = 1,18x - 0,11.

Conclusions: We conclude that HemosIL AcuStar ADAMTS13 Activity assay is a reliable test for the classification of ADAMTS13 activity. The results may be available in a very short time from sample arrival to the laboratory, the assay is fully automated and agreement with our current method was excellent at the low values required for diagnostic and therapeutic decisions. The availability of this assay may become an essential tool in the management of TTP patients, allowing for the early institution of caplacizumab or rituximab.

|CO7

VALUE OF KAPPA FREE LIGTH CHAIN AS A BIOMARKER IN CSF ANALYSIS FOR MULTIPLE SCLEROSIS DIAGNOSIS IN THREE CENTERS FROM PORTUGAL

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Introduction: The increase of the kappa free light chain in CSF of MS patients has been reported in several publications that had evaluate the value of K-index as a surrogate marker for the gold standard method for the determination of intrathecal synthesis of immunoglobulins, the detection of oligoclonal bands (OCB) in cerebrospinal fluid (CSF)(Leurs et al., 2020).

Goals: Evaluate the prognostic value of kappa free light chain and kappa index as biomarker for the results of the OCB tests, and asses its performance in the diferencial diagnosis of multiple sclerosis.

Methods: 199 paired CSF/serum samples from three different centers in Portugal, to which the OCB testing was requested were included. K-FLC was determined by turbidimetry (Freelite in Optilite, Binding Site). Statistical analysis was performed with the GraphPad Prism8 software.

Results: MS patients had a higher K-FLCCSF concentration and K-index (median:74,24) than the non-MS group (K-FLCCSF median: 3,77mg/L vs 0,3 mg/L) (K-index median: 74,24 vs 0,52). K-FLCCSF concentration in the samples with OCB positive was higher than in the samples with OCB negative results, 4,4 mg/L and 0,3 mg/L respectively, as well as the K-index, 70,24 and 0,52. A K-FLCCSF concentration <0,31 mg/L obtain in 86 samples (43,2%) showed a NPV of 97,7% for negative OCB. ROC analysis of K-index values vs BOC retrieve an area under the curve of 94,6% and versus MS diagnosis of 94,9 %. Such results are well above the ones obtain for the IgG-Index vs BOC and MS diagnosis, 76,1% and 76,9% respectively.

The previous published K-index cut-off of 6,6 had a sensibility of 94,4% and a specificity of 83,4% for MS diagnosis, in line with the reported by the authors (sens. 93% and spec 83%)(Leurs et al., 2020).

Conclusions: Our findings confirm the prognostic value of K-FLC as a biomarker for BOC results and MS diagnosis and can be integrated in an algorithm for MS screening that can help to reduce the volume of OCB determinations.

|CO8

FIRST-TRIMESTER COMBINED SCREENING: ONE OR TWO STEP APPROACH?

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Introduction: The natural frequency of most common aneuploidies (trisomy 21, 18 and 13) at birth approximates to 6 per 1000 births amongst women without any form of antenatal screening.

Effective screening of those aneuploidies is provided by assessment of the combination of maternal age, fetal nuchal translucency (NT) thickness, and maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at first trimester of pregnancy – combined test (CT).

According to published data, CT strategy, where blood testing (BT) and ultrasound scan (US) are carried out in the same visit, achives detection rates (DR) of 94% at 11 weeks, 90% at 12 weeks and 83% at 13 weeks for a 5% false-positive rate (FP). In an alternate strategy with BT at 10 weeks and the measurement of NT performed at 12 weeks, estimated DR of 96% for 5% FP would be expected.

Although the DR of the two step approach is slightly higher, it can only be accomplished if the timing of both exams are fulfilled.

Description: In our hospital there are two strategies for CT: one with BT and US carried out in two separate visits (BT done until 10 weeks and US at 12 weeks) and a second strategy with both performed in the same visit at 12 weeks.

The aim of this study was to verify whether the CT protocols were being performed at the recommended times.

A retrospective search of the database Astraia® was done to identify all singleton pregnancies in which CT was carried out from 01/01/2017 to 30/06/2019. A total of 5956 singleton pregnancies were identified. As much as 73.2% screenings were performed in a single visit, while the remaining 26.8% were performed in two visits. Only 42.1% of screenings performed at a single visit were done at 12 weeks. Among the two stage screening, only 14.5% were performed at the appropriate timing.

Discussion: Our study revealed that for both protocols the screening time was not being fulfilled, especially for two-stage screening. Therefore, the advantage in terms of detection rate is eroded by the increased non compliance with the additional step.

The authors propose the review of screening protocols with the implementation of screening in only one visit, strictly scheduled at 11 weeks.

|CO9

GENOTYPE AND PHENOTYPE – NOT ALWAYS A PERFECT MATCH IN BLOOD GROUPS

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Background: The ABO blood group system was the first system discovered, in 1901. However, variants due to the heterogeneity of A, B and O alleles still represent a challenge for immunohematologists, even with the advances in molecular methods.

Case Report: A G3P2A1 60-year-old woman with a uterine prolapse was proposed for a hysterectomy. A pre-operative type and screen was requested and a discrepancy was observed in the determination of ABO group. At room temperature, using Bio-Rad "DiaClon ABO/D" cards, no agglutination of the patient's red blood cells (RBC) occurred with anti-A and anti-B test sera. A very weak (<1+) agglutination was observed with anti-AB test serum. In the reverse typing, anti-B isoagglutinins were detected (4+), whereas anti-A1 isoagglutinins were not. Absence of anti-A2 isoagglutinins was also verified. At 4ºC, a very weak (<1+) reaction was observed with A1 RBC and patient's serum. Both direct and indirect antiglobulin tests were negative.

A weak subgroup of A was suspected and the ABO genotype was determined, using a sequence-specific primer polymerase chain reaction (SSP-PCR). The genotype ABO:O1A2 was determined, predicting an A2 phenotype. However, this phenotype typically presents a clear agglutination of patient's RBC in the presence of anti-A and anti-AB test sera, not consistent with the findings of this case.

Discussion: In ABO blood group system, the genotype/phenotype correlation may not be as straightforward as expected. The most acceptable hypothesis seems to be the presence of an allelic variation, for which the primers of SSP-PCR were not designed, that leads to a lower N-acetylgalactosaminyl transferase activity, decreasing the antigen H to A transformation and explaining the lack of agglutination of patient's RBC in anti-A reagent. Only DNA sequencing could confirm this theory, but the evaluation of A and H substance in the saliva and the analysis of the behavior of patient's RBC in presence of anti-H lectin could provide some clues.

|CO10

REFRACTORINESS TO PLATELETS TRANSFUSION: IMMUNE OR NON-IMMUNE?

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Background: Platelets transfusion can prevent and treat hemorrhage. However, some patients fail to produce a satisfactory response when transfused with platelets, which leads to an increased risk of morbidity and mortality.

Results: Woman, 75 years-old, G0P0, with auricular fibrillation, heart failure and a myelodysplastic syndrome (MDS), diagnosed in 2011 and resistant to lenalidomide since 2021, was hospitalized for decompensated heart failure. Seven days after admission, she presented with severe thrombocytopenia (9000 platelets/mm3) and a platelet transfusion was needed. Refractoriness to a pooled platelets transfusion was observed: 24h after transfusion the platelet count was 10000/mm3. Because of her medical history, decompensated heart failure and hepatosplenomegaly (22cm and 15.5cm, respectively), a non-immune refractoriness was assumed. For the next five days the patient received three additional platelet concentrates (pooled and apheresis) without platelet yield. The platelet count before and immediately after the next transfusion was obtained. The patient had a platelet count of 9000/mm3 both before and 30 minutes after a platelet apheresis transfusion was completed, suggesting an immune refractoriness cause. The Capture-P® Ready-Screen® was performed and an antibody anti-HLA class I was found. She had a previous history of red blood cells transfusion. After that, irradiated ABO and HLA-matched apheresis platelets were transfused with a better platelet yield. Nevertheless, the patient deceased on the 47th day of hospitalization due to terminal heart failure and progression of MDS.

Conclusions: The platelet count obtained immediately and 24h post-transfusion are used to establish the diagnosis of platelet refractoriness and to help determine the cause. In non-immune mechanisms, the platelet count initially rises, but afterwards platelets are consumed. In contrast, in alloimmune refractoriness, the platelet count never rises. Our patient had both non-immune and alloimune refractoriness, so it was important to avoid the implicated platelet antigens, but also to manage the underlying cause of platelet consumption. This case report is an example of how important is the multidisciplinary work between the patient's physician and the transfusion medicine laboratory.

|CO11

EVALUATION OF THE SYSMEX UF-5000 ANALYSER FOR SCREENING OUT URINARY TRACT INFECTION AND THE IMPACT OF BORIC ACID ADDITION TO URINE SPECIMENS

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Introduction: Urinary tract infections (UTIs) are an important health care problem for both hospitalized and community patients. Therefore, a rapid screening of UTIs is important to reduce unnecessary urine culture.

Objective: In this study we evaluated the diagnostic performance and accuracy of the Sysmex cytometer UF-5000 to screen out UTIs and the impact of boric acid (BA) addition to urine specimens.

Material e Methods: A prospective study was performed from 1st November to 31st December of 2020. A total of 269 urine samples were evaluated. The samples were collected in sterile urine cups. At the delivery time of the urines at the health care centers, a sample was taken from each urine into a new sterile cup with boric acid (BA) and then sent to the laboratory. On all the samples, a standard quantitative urine culture was performed. Cultures with growth ≥105 CFU/mL (colony forming unit per milliliter) were considered positive. Furthermore, all the urine samples were used for the UF-5000 analysis. The diagnostic accuracy of UF-5000 was examined for a range of cut-offs for the bacterial (BACT) and leukocytes (WBC) count. The best cut-off point was obtained from the ROC curve analysis to maximize the negative predictive value (NPV) and the best screening rate. Statistical analysis was performed with the software GraphPad Prism (version 5.0). The level of significance was set at $p<0.05$.

Results: The results showed no statistic difference between median values of BACT/mL in urine samples with and without BA, 306.9 [16.4; 27443.3] vs 147.4 [16.7; 7591.3] respectively. This fact can be justified by the prolonged time until the BA addition to the urine samples in primary health care centers. However, maximum performance was obtained for a cut-off of 71.5 BACT/mL, with NPV of 98.8% and a screening rate of 42% in urine samples with BA, and with 98.3% sensitivity (SEN) and 60.4% specificity (ESP). Regarding urine samples without BA the best cut-off was 181.5 BACT/mL, with a NPV of 96.5%. In both samples, WBC was not the best variable to be considered for screening.

Conclusion: This study demonstrated that UF-5000 can represent a valid tool for rapidly ruling out UTI, thus decreasing the turnaround time in the laboratory, with excellent sensitivity and very high NPV. Additionally, this can reduce in 42% the urine cultures, and thus the costs.

|CO12

FREE LIGHT CHAIN ASSAY AS A TOOL FOR EVALUATING DIALYSIS PERFORMANCE IN MULTIPLE MYELOMA PATIENTS WITH SEVERE KIDNEY INJURY

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Introduction: Severe kidney injury (mainly due to light chain deposition, cast formation and tubular obstruction) is observed in up to 20% of multiple myeloma (MM) patients, of which up to 5% need dialysis. High cut off dialysis may reduce the levels of circulating monoclonal free light chains (FLC).

A sustained reduction in FLC (through chemotherapy and hemodialysis) has been associated with the recovery of kidney function and better survival.

Objective: To evaluate the effectiveness of hemodiafiltration with ultrafiltrate regeneration (coupled with an adsorption filter – HFR SUPRA®, Medtronic) in reducing FLC (in serum and ultrafiltrate).

Materials and Methods: MM patients needing dialysis during 2020 and 2021 were included (n=6), with ages 46-79 years and the involved FLC being kappa (n=3) and lambda (n=3). Serum FLC before and after dialysis was determined by immunoturbidimetry (Freelite®, The Binding Site). In 2 patients (1 kappa; 1 lambda), FLC was also determined in the ultrafiltrate, pre- and post-adsorption filter.

Results/Discussion: In all patients, the dialytic technique effectively removed serum FLC (average rate of removal per patient per session: 35%, 51% and 55% for kappa; 20%, 22% and 28% for lambda). The lower rates for lambda may be due to different conformation (dimeric/polymeric) and higher molecular weight. Higher percentages of removal may not be attainable due to in vivo serum replenishment from extravascular compartment.

Measurements in the ultrafiltrate (pre- and post-adsorption filter) showed an effectiveness of nearly 99% in the beginning of dialysis for both kappa and lambda FLC. Approaching the end of the session, a filtration of 93% was obtained for kappa FLC, while a drop to 52% was observed for lambda FLC. Filter saturation could account for this variation, in particular for lambda FLC. Although ultrafiltrate is not a validated matrix for Freelite®, this data seems plausible.

Conclusion: FLC measurement in serum and ultrafiltrate of MM patients may help to assess the performance of dialytic techniques (in particular that with an adsorption filter) and to decide on the number of sessions or filter changes needed. Our sample was very limited, partly due to the relative rarity of renal support in MM. Multicentric studies may be the only way to produce more robust evidence in this context.

|CO13

COMPARISON BETWEEN MULTIPLEX PCR PNEUMONIA PANEL AND MICROBIOLOGICAL CULTURE IN LOWER RESPIRATORY TRACT INFECTIONS

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Background: Pneumonia is common in intensive care unit (ICU) patients. The conventional diagnosis includes cultural methods, in which the results can take 24 to 72 hours. Multiplex polymerase chain reaction (PCR) assays detecting a broad panel of bacterial and viral agents have the potential to shorten this gap between diagnosis and targeted treatment to less than two hours.

Objectives: The aim of this study was to compare BIOFIRE® FILMARRAY® Pneumonia Panel plus (BF-PP) results with microbiological culture.

Material and methods: A retrospective study of BF-PP tests made between April 2020 and February 2022 was performed. These results were compared with conventional culture and the agreement between the two methods was evaluated.

Results: A total of 188 samples from 153 patients were included in the study.

There were 98 samples (52.2%) with a positive result on the BF-PP.

On the negative BF-PP group, 56 samples (62.2%) had a negative cultural test, 29 (32.2%) and 5 (5.6%) had, respectively, cultural isolation of agents not included and included in the BF-PP.

Of the samples with a positive result in BF-PP, a single agent was identified in 52 samples, two agents in 23, three agents in 17 and four agents in 4 samples.

There were 28 samples (28.6%) with entirely concordant positive result. Microbiological culture was negative in 21 samples (21.4%) with positive BF-PP. Fungi or bacteria not included in the panel were isolated in 19 samples (19.4%). There was partial agreement (positive culture for some agents in BF-PP) in 28 samples (28.6%). In 2 samples (2.0%) an agent was isolated in culture, not identified by BF-PP.

The positive percentage agreement between the BF-PP and the culture was 91.5% (82.5 - 96.8) and the negative percentage agreement was 96.5% (95.7 – 97.1).

Conclusions: BF-PP is a useful tool for quick diagnostic of lower respiratory infections in ICU patients. However, it does not replace culture because of pneumonia agents that are not included in the BF-PP. In addition, a comprehensive antibiotic susceptibility test is only possible through culture methods.

BF-PP has high agreement with culture. The discrepant results can be explained by the greater sensitivity of molecular methods, the antibiotic therapy prior to specimen collection and the fastidious nature of certain bacterial agents.

|CO14

ATYPICAL HEMOLYTIC UREMIC SYNDROME: A CASE REPORT

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Introduction: Atypical hemolytic uremic syndrome (aHUS) is a rare complement-mediated disease, affecting 1-2 person per million, characterized by a triad of thrombocytopenia, microangiopathic haemolytic anemia and acute renal failure. Around 50-60% of the cases are associated with deficiencies of the complement regulatory proteins, including mutations in the complement factors H and I. It shows a severe course leading to end stage renal disease, so an early recognition is essential. The differential diagnosis includes typical HUS (associated with Shiga toxin) and thrombotic thrombocytopenic purpura (associated with ADAMTS13). Complement study, kidney biopsy and genetic sequencing help to do the diagnosis. The only specific treatment currently available is the eculizumab, a monoclonal antibody (Moab) anti-factor C5.

Case: A 37-year-old caucasian female presented to the emergency department with a 6 days history of cephalea, nausea and vomiting. She was found to have acute kidney injury (serum creatinine 7.53 mg/dl); proteinuria (300mg/dL), anemia (Hb 7.2g/dL), elevated LDH (1124U/L), low levels of haptoglobin, and slight thrombocytopenia (101.000/µl). Peripheral smear showed schistocytosis and coombs test was negative. She started plasmapheresis and haemodialysis. Further studies excluded infections and ADAMTS13 deficiency (antibodies negative and normal activity). Kidney biopsy revealed trombotic microangiopathy involving glomeruli and small vessels. Genetic study showed a heterozygotic missense mutation in factor H, in exon 22 (c.3562 A>G, p. Lys1188Glu), corroborating the diagnosis of aHUS. Eculizumab was started. Complete recovery was achieved with suspension of haemodialysis 2 months later. Nowadays maintains the Moab, showing clinical stability and response to therapy with alternative complement assay almost absent (0.3% NR:30-113).

Discussion: This case report highlights the importance of the prompt diagnosis of aHUS, in the presence of the triad mentioned above, since Shiga toxin or ADAMTS13 autoantibodies /deficit were ruled out. The complement study was not done in this case, but it could be helpfull and should be ordered. The genetic analysis, although more arduous and time consuming, sets the definite diagnosis. The functional complement assays are very important for monitoring treatment efficacy.

|CO15

EURACHEM/CITAC AQA 2021: ASSESSMENT OF PERFORMANCE AND UNCERTAINTY IN QUALITATIVE TESTS IN THE MEDICAL LABORATORY

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In the COVID19 pandemic, the design and development of virological screening tests and the evaluation of their performance were emphasized. Harmonizing best practices in this field is critical for binary result trueness.

This presentation aims to introduce the EURACHEM / CITAC guide "Assessment of performance and uncertainty in qualitative chemical analysis" (AQA 2021) from the perspective of the medical laboratory. The document was published on November 11, 2021, and it can be downloaded free of charge from the EURACHEM website. EURACHEM's Qualitative Analysis Working Group includes medical laboratory peers; Elvar Theodorsson, M.D., Ph.D., Professor of Neurochemistry, Linköping University, Sweden, and Paulo Pereira, Ph.D., Postdoc Senior Researcher of the Portuguese Institute of Blood and Transplantation, Lisbon, Portugal.

This guide reviews and introduces important principles in qualitative performance assessment. The evaluation is mainly based on Bayesian probability, through clinical sensitivity and specificity, from the perspective of the medical laboratory. The guide also reviews the agreement of binary results for cases where the condition is unknown, such as unknown diagnosis. The predictive values are reviewed if we consider the physician's view (clinical decision). An important concept introduced in this guide is the uncertainty of proportions, such as clinical sensitivity and specificity uncertainty. The uncertainty interval is estimated based on the 95% confidence interval principle. We can interpret it as the measurement of the chance of a given probability happening.

The guide is designed to apply to various areas of chemistry, including qualitative testing in the medical laboratory. The example of the performance assessment of an RT-PCR SARS-Cov-2 RNA test is a mere application that appears in the guide. It can be replicated for all qualitative tests with binary nominal quantities, such as true/false, positive/negative.

|CO16

CO-INFECTION WITH SARS-COV-2 AND INFLUENZA A VIRUS IN CHILDREN

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Introduction: The current COVID-19 pandemic imposed a number of hygiene measures unprecedented in history. Since the beginning of the COVID-19 pandemic, a decreased incidence of many viral infections has been reported in children. But in early 2022, influenza A virus infection began to be identified. With the lifting of mitigation measures, the flu virus increased circulation, and the concern of the emergence of co-infection with SARS-CoV-2 (Coviflu) arose.

Objective: To characterize Coviflu cases in children.

Methods: From 1st January to 10th of March were analysed 492 swabs of patients admitted in paediatric urgency room of Centro Hospitalar do Médio Tejo. Multiplex PCR were performed using BIOFIRE® Respiratory 2.1 plus Panel (bioMérieux, USA). SARS-CoV-2 variants were identified using Applied BioSystems (USA) protocols in CFX-96 (BioRad, USA). Ómicron sub-lineage BA.2 were identified due absence of del Δ69-70 mutation.

Results: From 492 patients under 18 y/o, 25 (5%) presented Influenza A virus type H3 (AH3), and 4 (0.8%) had also SARS-CoV-2 co-infection. Patients with Flu A virus, were mostly men (n=16; 64%). Coviflu infection were observed in 3 men (A, C, D), aged with 1, 6 and 9 y/o, and 1 female (B), aged 15 y/o (Table 1). Patient A revealing slight fever (37.7 ºC), cough, and rhinorrhea were also co-infected with adenovirus, and coronavirus 229E. Patient B showing cough, fever (39.2ºC), vomiting, and muscle pain; and patient C with fever (38.7ºC) and headache had only identified with Coviflu. Patient D with fever were co-infected with

coronavirus 229E, and Rhino/Enterovirus too. No internment was necessary. All had SARS-CoV-2 Ómicron variant, but patient A and B the BA.2 sub-lineage, and patients C and D BA.1 sub-lineage.

Conclusions: This work showed the importance of simultaneous detection of Coviflu. This follow-up is important to see if the flu virus exacerbates the symptoms of SARS-CoV-2. Also, monitoring the relief of mitigation measures at the same time will make it possible to know if the circulation of the Flu virus has just been postponed, or if SARS-CoV-2 is replaced the same way that SARS-CoV-2 replaces the influenza virus in 2020, after implementing sanitary measures. To our knowledge this is first study characterizing Coviflu and SARS-CoV-2 variants identification. We call on the medical community to be aware and take COVID-19 into account as a potential diagnosis even in patients with other viral causes, especially in epidemic areas.

|CO17

IMMUNE RESPONSE AFTER FULL SARS-COV-2 VACCINATION

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Introduction: The global pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has been the greatest public health challenge of the last century. In this context, several vaccines were developed to combat SARS-CoV-2. In Portugal, vaccination began in December 2020.

Objective: This study aimed to estimate SARS-CoV-2 antibody levels after a full vaccination course.

Materials and Methods: An evaluation of acquired immunity by determination of anti-spike glycoprotein immunoglobulin levels, via Chemiluminescent Microparticle Immunoassay (CMIA) (Abbott Architect i2000SR), in serum samples collected from healthcare workers was conducted. Antibody levels were determined at 3 timepoints: Pfizer® vaccine (PF): T0 – day of 1st dose administration; T1 – 21 days after the 2nd dose; T2 – 6 months after T0. AstraZeneca® vaccine (ATZ): T0 – day of 1st dose administration; T1 – 1 month after the 2nd dose; T2 – 6 months after T0. An IgG result >50 AU/mL was considered positive for immunity.

380 participants were included: 213 were vaccinated with PF, 167 were vaccinated with ATZ. Exclusion criteria: positive immunity at T0 (n=21), negative immunity at T1 (n=2) or T2 (n=4), and participants that received different brands for each dose (n=15). At each timepoint, the median and respective quartiles [P25; P75] were calculated using GraphPad Prism (version 5.0). The significance level was set at $p<0.05$.

Results: The average age of the study population was 41.9 ± 10.8 years, and 87.2% were females. The PF group had a median immune response of 15645 [10514;23130] AU/mL at T1, and 1509 [922.4; 2290] at T2. The ATZ group had a median immune response of 1060 [614.4; 1919] at T1, and 399.7 [186.9; 769.8] at T2. A statistically significant increase of antibodies was found in both groups ($p<0.001$) between T0 and T1, showing a clear immune response to vaccination. However, the PF group had a notably larger increase than the ATZ group (15645 vs 1060, $p< 0.001$). At T2, both groups showed a marked drop in antibody count, with PF once again showing superior performance (1509 vs 399.7, $p<0.001$).

Conclusion: This study showed a higher seroprevalence of SARS-CoV-2 specific antibodies in participants vaccinated with PF.

|CO18

PORPHYRIA CUTANEA TARDA – TEN YEARS OF FOLLOW-UP AT A TERTIARY HOSPITAL

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Introduction: Porphyria Cutanea Tarda (PCT) is the most common porphyria, due to an insufficient/altered uroporphyrinogen decarboxylase (UROD) enzymatic activity. Such deficiency leads to an accumulation of porphyrins in the liver. The diagnosis must be considered in patients with photosensitivity and cutaneous bullae.

Aim: A ten years evaluation of epidemiology, triggers and treatment of PCT in a tertiary hospital.

Material and Methods: Retrospective study of 31 patients with PCT conducted between January 2011 and December 2021. Parameterized research using the laboratory's computer system to analyze the values of ferritin, porphobilinogen, uroporphyrin, coproporphyrins and protoporphyrins, HFE gene and UROD gene. Age, gender, clinical presentation, laboratory tests at diagnosis, triggers and treatment were obtained from hospital database.

Results: 71% of patients were male and 29% female, with a mean age of 52±12.9 years. 58% of patients performed a search of HFE gene: 11% were homozygous for H63D, 11% were heterozygous for C282Y, and 22% were heterozygous for H63D. Only in three patients was performed the search for the mutation in the UROD gene and only one was heterozygous for c942G>A. At the diagnosis, the laboratory results were: ferritin 566 ± 371ng/ml, uroporphyrins 1163 ± 1138ug/24h, coproporphyrins 770 ±362ug/24h and porphyrins 1739 ±1288ug/24h. In the exacerbations the most frequent precipitating factors were: alcoholism (39%), Hepatitis C virus (HCV) (16%), estrogen use (3%), HIV associated with HCV and alcohol use (6%). Patients with HCV are those with most elevated mean values of uroporphyrins (3655.3±909ug/24h).

Regarding the treatment, 29% of patients were not submitted to any type of therapy, 25.8% underwent phlebotomies and only one patient was treated with plaquinol. 70% of patients presented a significant reduction of ferritin upon treatment (-68.9±19.1%).

Discussion: In this retrospective study, 31 patients with PCT were studied. PCT usually has a sporadic presentation, affecting more men in the 5th decade of life. The main triggers of exacerbations are alcoholism and HCV. The most used therapy was phlebotomy, with good response and decreased ferritin.

|CO19

CAN WE RELY ON A 1973 FORMULA TO CALCULATE ALBUMIN-ADJUSTED CALCIUM?

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Introduction: The gold standard to assess calcium status is the measurement of ionized calcium. Nevertheless, there is still significant clinical demand for the determination of albumin-adjusted total calcium. This is achieved using a mathematical equation (method-dependent) that includes seric total calcium (Ca) and seric albumin concentration (Alb).

Objective: The aim was to calculate and validate the equation that best reflects our population, using our laboratory methods.

Materials and Methods: A retrospective study was done based on simultaneous Ca and Alb measurements of 9165 patients. Both measurements were performed using the Abbott Architect ci8200 by Arsenazo III (Ca) and bromocresol green (Alb) methods.

Exclusion criteria: age < 18 years, creatinine > 2.26 mg/dL, Alb < 2.0 g/dL or > 5.0 g/dL, total Ca > 12 mg/dL, parathyroid hormone, alkaline phosphatase and/or alanine transaminase above the reference value.

The population studied was randomly divided into two cohorts: derivation cohort with 75% of the samples (nd=6874); validation cohort with 25% (nv=2291). Simple linear regression associating Ca and Alb was constructed from the data in the derivation group, which was subsequently validated in the validation group. Our equation performance was later compared with the most frequently used equation (Payne's formula) Adjusted[Ca](mg/dL) = Total[Ca](mg/dL) + 0,8(4,0 - [Alb](g/dL), in 1354 subjects with hypoalbuminemia (Alb < 3,4 g/dL).

Results: From the analysis of our data, we obtained the equation Adjusted [Ca](mg/dL) = Total [Ca](mg/dL) + 0,803 (3,979 - [Alb](g/dL)), which showed good internal validity (adjusted r2 shrinkage = -0,022).

When comparing the Ca status values obtained in individuals with hypoalbuminemia (n=1354) using the two equations, we found that only 14 were not in agreement (weighted kappa = 0.99), revealing very good agreement.

Conclusion: The equations are in accordance, although the Ca assay method differs between them. There is a paradigm shift regarding Ca measurement methods, which does not seem to affect the results in determining Ca status using these formulas. However, when focusing on improving the quality and the clinical relevance of the laboratory results, it is extremely important to assess whether the "standard formulas" are adjusted to our population.

|CO20

CASE REPORT - IS IT ONLY MALARIA?

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Introduction: Malaria is one of the main parasitic diseases in the world, potentially fatal in the absence of timely and targeted treatment. It is caused by Plasmodium spp. and its severe form is caused almost exclusively by P. falciparum.

Clinical case: A Cape Verde 13-year-old female, resident in Nigeria for 1 year, has been in Portugal for 15 days and went to the emergency department in January 2022 presenting fever with 3 days of evolution, associated with generalized headache, vomiting and back pain, with gait limitation. In the last 12 months, she had 2 episodes of malaria, the last one in October, with no other relevant history.

The blood count showed normocytic normochromic anemia, leukopenia with lymphopenia and thrombocytopenia. She presented with liver function aggravation and the increase of D-Dimers, CRP, PCT and VS. Blood culture and urinary sediment were negative. She showed positive Paul-Bunnel, positive blood smear for Plasmodium (1.5% parasitemia) and positive P. falciparum antigen. Treatment with artemether/lumefantrine was initiated, considering Nigeria as an endemic area of chloroquine resistance.

During hospitalization, analytical aggravation of anemia, leukopenia, thrombocytopenia and hepatic markers were observed, having been transfused with hemoderivatives and transferred to CHUSJ due to hemodynamic instability.

Immunological study was positive for VCA EBV IgG and EBNA, IgM Mycoplasma, anti-Borrelia B.Burgdorferi IgG and IgM, and IgG antibodies to Dengue virus. Confirmation of anti-Borrelia by Western-Blot was IgG and IgM positive.

To confirm these findings, it was performed a study of Plasmodium, Borrelia and EBV by RT-PCR, which was positive only for Plasmodium spp.

Discussion: A cross-reaction happens when an antibody binds to an antigen for which it was not specifically produced, since it has correlated structures. Thus, it is possible for antibodies to react to antigens without prior exposure.

The presence of vectors for Borrelia in Nigeria and Cape Verde is not described in the literature. The most frequent cause for this type of findings is intravenous gammaglobulin therapy, which was not observed.

A diagnosis comprehends the laboratory findings and the clinical diagnostic methods, such as clinical markers, physical examination and imaging techniques. Most important is a consideration of the final clinical picture to decide if the laboratory results fit the clinical and endemic context of the patient.

Oral Communcations – Rapid Pace

|CR1

INFECTIVE ENDOCARDITIS CAUSED BY ABIOTROPHIA DEFECTIVA - CASE REPORT

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Introduction: The diagnosis of Infective Endocarditis (IE) is made through clinical suspicion, the presence of positive blood cultures (BC), a new heart murmur and an echocardiogram with evidence of vegetation. The main agents are Staphylococcus aureus, Streptococcus viridans group and enterococci.

Abiotrophia defectiva is a bacterium that can have a pleomorphic appearance in Gram stain and has a difficult growth in the usual culture media. It is part of the oral commensal flora and the secretion of exopolysaccharides and the ability to adhere to fibronectin make it have a particular affinity for the endovascular tissue and may cause endocarditis.

Case report: A 36-year-old woman with a diagnosis of bicuspid aortic valve in childhood, presents with complaints of asthenia, anorexia and myalgia with 5 months of evolution. Cardiac auscultation showed grade III / IV aortic systolic murmur. Analytically, she had hypochromic microcytic anemia, 9,000 leukocytes / uL, PCR of 155 mg / L and ProBNP of 5873 pg / ml. A transthoracic echocardiogram was requested, which revealed a bicuspid aortic valve with limited opening amplitude and an ecodense image at this level, mobile, with 13x12mm, which may correspond to vegetation.

Three BC were collected, which were positive after 3 days. In the direct exam stained by Gram pleomorphic gram positive bacilli were observed. A strain of Abiotrophia defectiva grown on blood agar and it was identified using Maldi-TOF MS. The patient completed an antibiotic cycle with ampicillin and gentamicin and aortic valve replacement was performed, without microbiological isolation in the sample.

Discussion/Conclusion: Abiotrophia defectiva is a rare but important IE agent, with potentially serious consequences. In most of the cases described, there is an underlying cardiac pathology, as in this case the presence of a bicuspid aortic valve. The clinical course is usually slow. The bacteria has a slow growth, so it is important to increase the incubation time of BC and to prolong for at least 72h the incubation of the media in an atmosphere containing CO2, in the attempt to recover the agent.

This case shows the importance of clinical suspicion, in cases of indolent presentation of EI, as well as a correct diagnosis with a timely identification of the agent for an appropriate antibiotic and surgical treatment.

|CR2

NONCONFORMITIES IN FIRST TRIMESTER COMBINED SCREENING TEST REQUESTS – DEALING WITH UNACCOMPLISHED GOALS

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Introduction: Screening for trisomy 21 using the Combined Test (CT) is part of routine care in the first trimester of pregnancy. However, some apparently minor errors at the time of screening request can seriously compromise the whole process.

Description: The aim of this study was to retrospectively review nonconformities (NC) associated with CT requests that precluded risk calculation.

CT requests performed between 01.01.17 and 30.06.2019 were reviewed using eDEIA-Lab® software and risk assessment software Astraia.

During the study period there were 6013 CT requests, 179 (3%) with no risk calculation as result of NC.

Inappropriate test request was the most common NC (39.1%). This mainly included repeated CT requests (67.1%) and early pregnancy loss at time of screening. Repeated requests were often accompanied by new blood samples, despite biochemical markers had already been determined, and 30% already had a CT result.

The second most common NC (30.7%) was late sample collection (blood sampling after 14 weeks). In 54.5% of such cases biochemical testing and ultrasound scan (US) were carried out in two separate visits.

Lack of fetal nuchal translucency (NT) measurement at adequate gestational age (crown rump length 45-84mm) was also a common NC (27.9%). Pregnant women failed to show up the scheduled visit in 32% of cases. No information was available to further clarify the remaining cases.

The least common NC (2.2%) was early blood sampling (before 8 weeks).

Discussion/Conclusion: Inappropriate request was the most common NC resulting in incomplete CT. When accompanied by a new blood sample, repeated requests can go undetected prior to sample processing, rising laboratory costs.

Late sample collection and lack of NT value are most worrisome, as CT becomes impossible. Our results show the importance of early US to assess gestational age, allowing for appropriate scheduling of blood collection/NT measurement and detection of early pregnancy loss.

Although higher detection rates can be achieved by biochemical testing at 8-10 weeks and measurement of NT at 11-12 weeks, a likely increased non-compliance with the second step may erode its potential advantage. We believe that if blood test and US were done in the same visit (ideally at 11 weeks) many NC would be avoided, with no negative impact on screening performance.

|CR3

LIPAEMIA WITHOUT LIPAEMIA – CONCEALED MONOCLONAL PROTEIN?

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Introduction: The determination of serum indexes, such as haemolysis, icterus and lipaemia (HIL status), is of great importance to the identification of the interferences present in a sample. These represent a significant source of error in the determination of analytes, with all the associated clinical and laboratorial consequences.

The automatic determination of the HIL status varies according to the systems used. It is measured by spectrophotometry using the absorbance spectrums of haemoglobin (340-580nm), bilirubin (~460 nm) and lipaemia/turbidimetry (>400nm). Interference might be caused by the presence of other elements in the sample that are detectable on the same wavelengths, accounting for possible loss of specificity.

Description: A sample originating from a hospital ward was processed using our department's automated laboratory system for clinical chemistry analysis, comprising of several Alinity c (Abbott). It presented with an estimated lipaemia index of >200. However, the macroscopic appearance of the serum did not match the determined lipaemia index, being colourless and clear. It was hypothesised that a monoclonal protein was present in the sample, so the serum immunoglobulins and light chains were measured. An IgM concentration of 10.48 g/L was obtained (RV 0.33-2.93 g/L) and a K/λ relation of 1.57 (RV 1.30-2.61). The protein electrophoresis revealed the presence of two monoclonal proteins in the gamma region (0.22 g/dL and 0.20 g/dL) and its characterization by immunofixation identified an IgM lambda monoclonal protein.

Discussion: Monoclonal proteins and polyclonal immunoglobulins are known causes of pre-analytical interference, increasing the lipaemia index. Their presence may be shown by a discrepancy between the lipaemia index reported by the automated analysis systems and the visual evaluation of the sample's turbidity.

In a routine laboratory situation, with automated laboratory systems, the direct visualization of the samples with high lipaemia index levels may be decisive for the identification of unexpected analytical abnormalities, such as the presence of monoclonal proteins.

|CR4

DETERMINATION OF PREANALYTICAL UNCERTAINTY FOR SERUM LIPID METABOLISM ANALYTES

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Introduction: In the Medical Laboratory, uncertainty on the result may arise from different sources, including pre-analytical, analytical and postanalytical phases. Accounting the highest percentage of error in the total analytical process, preanalytical phase is often neglected as a source of uncertainty. The assessment of preanalytical uncertainty is fundamental to determine how these sources of variation can affect the final result.

Objective: As part of a long term project, designed to assess all the preanalytical phase, the aim of the study was to determine the combined (uc) and expanded (U) uncertainty associated to preanalytical phase variability, specifically venous puncture, processing delay, refrigeration, freezing, transport and lack of homogenization, on four lipid metabolism analytes in serum samples: Triglycerides (Trig), Cholesterol (Chol), High-Density Lipoprotein Cholesterol (HDL) and Low-Density Lipoprotein Cholesterol (LDL).

Material/Methods: Blood was collected from each arm of 56 volunteers into 5 serum-separation tubes. The data pairs for each procedure were evaluated according to laboratory standard conditions and the experimental alternative and converted into total coefficient of variation (CV) values. Standard preanalytical uncertainty for each variable were obtained by subtracting the analytical CV from the total CV. Combined uncertainty (uc) was determined by incorporation of standard uncertainty for each variable. The samples were analysed on Siemens Advia®1800 Chemistry System.

Results: Expanded uncertainty (U) for Trig and Chol were 4,87% and 0,10%, respectively. Freezing conditions affects all analytes almost equally. The preanalytical variable transport did not have an impact in none of the studied analytes. Triglycerides was the only parameter affected by puncture, refrigeration, processing delay and lack of homogenization.

Conclusion: Preanalytical uncertainty impacts the final result, adding variation. Knowledge of preanalytical factors affecting results should be considered in laboratory medicine. Thus, estimating preanalytical uncertainty should be emphasized. In the future, ISO standard 15189, should indicate methodology on uncertainty estimation/calculation and reference tables should be created to compare uncertainty values.

|CR5

THE IMPORTANCE OF NEW METHODOLOGIES IN THE DIAGNOSIS OF HUMAN INFECTIONS – FIRST REPORTED CASE OF KERSTERSIA GYIORUM IN A TERCIARY HOSPITAL

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Introduction: Kerstersia gyiorum is a pathogen rarely isolated in humans. Infection often goes undiagnosed due to lack of knowledge and laboratory capacity to correctly identifying it.

Case report: Female, 86 years old, partially dependent with an history of hypertension, dyslipidaemia and melanoma diagnosed in 2016 on the left calcaneus, with surgical eradication of the lesion. No follow-up was performed after eradication. From 2019 on, patient had multiples visits to the Emergency Department due to new left lower limb (LLL) lesions and over-infection which resulted in a Dermatology consult in 2020.

Patient presented multiple erythematous nodular lesions and two large adenopathies in the left inguinal and axillary regions. PET revealed subcutaneous thickening of the LLL, compatible with metastasis. Several left inguinal and pelvic hypermetabolic adenopathies, compatible with secondary locations. B-RAF mutation study was negative. Patient was sent to the Palliative Care consult for pain and dressing management.

In January 2021 the patient returned to the Emergency Department due to an episode of lipothymia. CT was performed to screen for brain metastases and a wound swab was performed due to the presence of a greenish, foul-smelling exudate on the chronic lesions of the LLL.

Microbiological study showed Gram staining with polymorphonuclear leukocytes and gram-negative bacilli and isolated on blood agar by Maldi BiotyperTM (Bruker Daltonics, Germany), Kerstersia gyiorum and Pseudomonas aeruginosa. Patient initiated antimicrobial therapy, but shortly after was deceased due to SARS-CoV-2 infection complications.

Discussion / Conclusion: Kerstersia gyiorum is an extremely rare pathogen in human infections. It is most frequently reported in patients with chronic wound infections and underlying conditions. It can easily be misdiagnosed if proper diagnostic methods are not used. To the best of our knowledge this is the first reported case in Portugal.

The isolation of Kerstersia gyiorum was only possible due to the use of the MALDI-TOF MS - BruckerTM technology whose database is wider than the one previously used (MS-VITEKTM). This technology allows the identification of gram-negative, gram-positive, aerobic and anaerobic microorganisms, as well as mycobacteria and yeast cells, usually at the species level, with very good accuracy.

|CR6

FULMINANT BLASTOID VARIANT OF MANTLE CELL LYMPHOMA WITH LEUKEMIC PRESENTATION – A CASE REPORT

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Mantle cell lymphoma (MCL) is a B-cell non-Hodgkin lymphoma. According to the 2016 WHO classification of hematopoietic and lymphoid tumors, there are two major subtypes of MCL: classical and leukemic non-nodal. We report a case of a classical MCL, blastoid variant, with a markedly increased leukocyte count and very aggressive clinical course.

A 68-year-old man, previously healthy, presented with one-day evolution headache and odynophagia, and long lasting fatigue. Laboratory tests revealed markedly increased leukocyte count (669.00 x 109/L), anemia (red blood cell count: 2.77 x 1012/L; hemoglobin: 6.60 g/dL; mean cell volume: 93.50 fL; mean cell hemoglobin concentration: 25.50 g/dL), low platelet count (71.00 x 109/L), slightly increased inflammatory markers (C-reactive protein: 5.92 mg/dL), impaired renal function (serum creatinine: 3.90 mg/dL; blood urea nitrogen: 64.00 mg/dL), and elevated lactate dehydrogenase (1757 U/L) and serum uric acid (27.10 mg/dL). The peripheral blood smear revealed a spectrum of cells with cytological features suggestive of blasts. The diagnosis of acute leukemia versus lymphoma in leukemic phase was taken into account, and peripheral blood was promptly sent to immunophenotyping. The flow cytometric immunophenotyping suggested the diagnosis of MCL. Despite immediate supportive therapy, the patient's clinical condition deteriorated rapidly and he died 5 hours after the diagnosis.

MCL is considered one of the most aggressive lymphoid neoplasms with relatively short responses to therapy and frequent relapses in spite of early and intensive treatment strategies. The incidence of leukemic expression in MCL varies highly in different studies, but it seems to be a common feature. The blastic variant form of MCL (BV-MCL) is considered to have worse prognosis. Since cells have blastoid morphologic features, the differential diagnosis should include acute leukemia and chronic lymphoproliferative disorders, notably when peripheralized.

The laboratory is a key pillar in the diagnosis of MCL. The recognition of BV-MCL relies on cytomorphology examination, but correct diagnosis can be problematic if used alone. Immunophenotyping has become an integral part in the diagnostic workup, but, whenever possible, confirmatory molecular genetic tests and/or immunohistochemical studies should be performed.

|CR7

SIGMA METRICS ESTABLISHED ON THE NUMBER OF DEFECTS PER MILLION OPPORTUNITIES TO COMPUTE THE MEDICAL LABORATORY CAPABILITY INDEX

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Introduction: The sigma allows a more natural classification of the capability of the test to comply with the specifications. Performance below 3-sigma is related to inherently unstable and unacceptable processes. Sigma policy is oriented toward

eliminating defects and reducing variation. An empirically-based 1.5 sigma shift is introduced to the calculation to account for this real-world increase in process variation over time. Given this principle, a process that fits between the process mean and the closest specification limit in a short-term study will in the long term provide a shorter sigma, either because the process mean will move over time or because the long-term standard deviation of the process will be higher than that observed in the short term, or both.

Objective: Evaluate the laboratory´s capability index.

Materials and methods: mathematical model: DPMO = (n_defects*1000000)/(n_defect opportunities*n_units). Spreadsheet functions are used: sigma_DPMO = ABS((NORMSINV(DPMO/1000000))+1.5.

Results: Let us consider Lab A. Over 362 days, the laboratory tested 81,450 human samples (units). Results from 225 human samples were rejected (defects) due to out-of-control IQC results, i.e., 0.3% of defects. The number of opportunities for defects is three, representing the pre-examination processes, examination processes, and post-examination processes. There were reported 11 conformities (defects) associated with non-conforming results in the three phases. sigma_DPMO = 4.6.

Discussion: We suggest that DPMO-derived sigma metric equal to or higher than 4-sigma is referred to as ´´satisfactory process´´ – meets specification limits, from 3-sigma to 4-sigma as ´´capable process, but marginally´´ – the process will not tolerate a significant shift, and if lower than 3-sigmas as ´´unsatisfactory process´´ – the process is out of specification or about to happen. However, the study is based on long-term data, so 1.5-sigma should not shift the most accurate measurement. Therefore, 3.11-sigma is a more realistic expression of the capability of the test to classify qualitative results correctly. So, the Lab A process is classified as ´´capable process, but marginally.´´

Conclusions: The sigma using ´´the classical DPMO model´´ is the truth for a given period (retrospective study design) based on the number of defects per million opportunities. This approach is not an alternative to Westgard´s ´´sigma metrics´´ model, which focuses on assessing the performance of the assays.

|CR8

ACQUIRED FACTOR XI DEFICIENCY: CLINICALLY RELEVANT?

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Introduction: Plasma protein coagulation factor XI (FXI) is the zymogen of the coagulation protease FXIa, which contributes to physiological hemostasis. In FXI-deficient patients consistently prolonged activated partial thromboplastin time (aPTT) is observed and usually it is longer in plasma lacking FXI than in plasmas missing FVIII or FIX. Current laboratory methods are unable to assess bleeding risk in FXI-deficient patients, as the degree of bleeding tendency does not correlate with plasma FXI activity as measured by routine coagulometric aPTT–based assays.

Case Report: We present a case of 85-year-old female patient with markedly prolonged aPTT (81.9 sec, reference 30.0 sec) during routine preoperative coagulation assays before cataract surgery. Antithrombotic therapy usage, drug ingestion, liver dysfunction and sepsis were excluded. Clinical background revealed no bleeding manifestations and a comprehensive bleeding diathesis workup showed factor FXI levels severely decreased (FXI:C 6.10 %) and the presence of inhibitors (37.3 sec, reference 31.0 sec), inhibitor titer was not performed due to lack of blood sample. Immunosuppression with prednisolone during 11 weeks was accomplished and a slight insignificant increase of FXI (FXI:C 9.20 %) was observed. Cataract surgery was performed and no minor nor major bleeding occurred.

Discussion: Diagnosis of FXI deficiency is found incidentally as part of presurgical workup for a prolonged aPTT and rarely as a result of bleeding. A search for an inhibitor is mandatory in these circumstances, even if before surgery, no inhibitors could be detected. Most bleeding manifestations in patients with severe FXI deficiency and inhibitor are injury related. Some patients do not bleed, and in others, bleeding manifestations vary even for the same procedure.

|CR9

INTERNAL CONTROL OF BINARY ORDINAL QUANTITIES BASED ON LOSS OF CLINICAL SENSITIVITY: APPLICATION TO THE SCREENING OF INFECTIOUS DISEASES

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Introduction: The qualitative control may involve binary nominal quantities ("purely" qualitative, unrelated to another expression) or binary ordinal quantities (qualitative, but classified on an ordinal scale according to a cutoff point). This presentation considers the second type of quantities. In this case, the use of Levey-Jennings cards and "Westgard rules" (six rules) is a common practice. As has already been demonstrated, using these types of rules in this analytical method is a cause of a large number of false alarms. Other methodologies involve external quality assessment data (high heterogeneity of data), which contributes to (falsely) wide limits - there is a significant lack of sensitivity for detecting real errors. Quantitative logic should not be applied to this type of test. The problem in this type of test is the false results (biased results). Thus, we will focus on systematic error and positive samples with a low signal but systematically superior to the cutoff. The qualitative logic presented is based on the loss of clinical sensitivity and follows the best internal quality control practices.

Objective: To propose a methodology involving (allowable) loss of clinical sensitivity and analytical capacity index using the sigma.

Materials and methods: mathematical models: critical systematic error, SE_crit = [(x_QC - CO) / s_meas] - z; sigma metric, = SE_crit + z. The loss of sensitivity is expressed by the difference between the mean and the cutoff value. EZ Rules 3 software (Westgard QC, Madison, WI) is used to infer quality control design based on SE_crit or sigma.

Results: We will consider an immunoassay to screen for anti-hepatitis C antibodies. Cases 1, 2, and 3 have sigma greater than 6 for control materials with a concentration (S/CO) of 1.76, 2.24, and 3.16.

Discussion: The sigma metric suggests a simple rule, 1: 3s, and a low frequency of samples per run (n = 1; n = 2). This design has a high probability of error detection (p_ed> 0.90) and a low probability of false rejection (p_fr <0.05), which can be interpreted as an excellent sensitivity for the classification of true positives in samples with low signal.

Conclusions: It is essential to recognize that the control point is the concentration of the material. Concentrations much higher than 3 S / CO may not support fitness for purpose. The use of the "gray zone" will make this approach even more robust as it introduces a tolerance.

|CR10

COVID-19 SEVERITY ACCORDINGLY SARS-COV-2 VARIANTS

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Introduction: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has provoked coronavirus disease since 2019 (COVID-19), which ranges from asymptomatic or mildly symptomatic infections to severe pneumonia, respiratory failure and death. Many new variants of the SARS-CoV-2 have been denominated variants of concern/interest because of the greater risk they possess due to possible enhanced transmissibility and/or clinical severity, diagnostic and/or treatment failure. The pandemic, highly transmissible SARS-CoV-2 has indeed caused considerable morbidity and mortality and drastically changed our everyday lives. The purpose of this study is to evaluate the COVID-19 severity of the different SARS-CoV-2 variants in hospital admitted patients.

Methods: Between 16 of February 2021 and 6 of February 2022 there were identified variants of 71 positive samples of SARS-CoV-2, at the Centro Hospitalar Médio Tejo. Variants were identified using GSD Novatype SARS-CoV-2 ID (Germany) and Applied BioSystems (USA) protocols in CFX-96 (BioRad, USA). Ct values were performed accordingly manufacture. Severity of COVID-19 were classified accordingly NIH (2022), based in respiratory failure as Mild, Moderate, Severe, and Critical Illness.

Results: Thirty-two (45%) patients were sorted to COVID-19 ward and 39 (55%) to Intensive Care Unit (ICU). From the 71 patients admitted to the ward, 44 (62%) were identified with the Delta variant, 11 (16.9%) with Alpha and 11 (16.9%) with Omicron. The most patients [41 (57.7%)] admitted with Severity and Critical Illness have been identified with Delta variant, 9 (12.7%) with Alpha and 9 (12.7%) with Omicron. The analyses of the SARS-CoV-2 severity showed that 27 (44%) required invasive mechanical ventilation (IMV), which 1 was indicated for Extra Corporeal Membrane Oxygenation (ECMO). Thirteen (21%) required non-invasive mechanical ventilation (NIV) and 1 had no criteria for IMV. From the patients with IMV, 11 (40.7%) have died [8 (72.7%) Delta variant] and 10 (37%) were discharged. Twenty-three (52%) patients with the Delta variant were discharged and 14 (31.8%) have died, while 9 (75%) of patients identified with the Alpha variant were discharged and 3 (25%) have died.

Conclusion: Delta Variant was predominantly identified in admitted patients as well as in IMV need. This variant has also been associated with higher severity of COVID-19 outcome.

|CR11

CANCER DIAGNOSIS – WHEN THE COAGULATION STUDY IS THE KEY

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Background: Bleeding can be a manifestation of multiple clinical entities. The correct interpretation depends on requesting the appropriate study and integrating it correctly with the clinical findings, a process in which the multidisciplinary approach is crucial.

Case report: A 43-year-old woman underwent a surgical exodontia. In the following 2 weeks, she went back to the hospital for 4 times due to persistent oral bleeding. The transfusion of 2 units of packed red blood cells was needed. The patient also reported menorrhagia in that month. Three weeks after the procedure, she was admitted to the emergency department with cough, fever and severe asthenia, presented over the past 6 days. The physical examination revealed a peripheral oxygen saturation of 60%. An arterial blood gas test was performed, revealing a severe respiratory failure (pO2 33,5 mmHg), with hypocapnia (pCO2 24,9 mmHg). These findings, associated with atypical abnormalities of the chest X-ray, led to the diagnosis of pneumonia and antibiotic initiation. A coagulation study was also requested: prothrombin time 23 seconds (normal: 11,5-14,5), activated partial thromboplastin time 43,9 seconds (normal: 24,0-34,0). In front of this study and after investigation in the electronical process, the clinician of the Immunohemotherapy department decided to add fibrinogen assay to the requested study, considering the bleeding history. A fibrinogen level of 48 mg/dL (normal: 200-400) was determined. D-dimers >20 µg/mL (positive if >0.5) were also assessed, with a platelet count of 155000/mm3. A massive pulmonary embolism or a disseminated intravascular coagulation (DIC) were considered as the main diagnosis hypotheses. Thus, a thoracic computed tomography was performed, revealing a probable pulmonary neoplasia, with diffuse metastases (liver, bone and ganglia). A paraneoplastic DIC was, therefore, assumed as the most probable explanation to the coagulation study findings.

Conclusion: In this case, the investigation of the clinical history by the clinician in the laboratory and his active role in guiding the analytical study to be performed were fundamental to the establishment of the diagnosis, emphasizing the importance of multidisciplinary patient management to achieve the best possible integration of clinical and analytical findings.

|CR12

MICROSCOPIC HEMATURIA IN ADULTS

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Introduction: Microscopic hematuria is defined as the presence of more than 2 or 3 red blood cells (RBCs) per high-power field (HPF) confirmed on 2 or 3 separate urinalyses. It is a relatively frequent finding among the general population in most population based screening studies. Its main etiologies are usually benign, self limited and with a good prognosis. Despite this, urologic causes should always be excluded if risk factors are present. Renal causes should be suspected when dysmorphic RBCs are found in a urinary sediment, and further testing should be done if there is a concurring proteinuria and a declining glomerular filtration rate (GFR).

Clinical Case Report: We present the case of a 68-year-old woman with asymptomatic isolated microscopic hematuria (RBCs 5-9/HPF) found in a routine urine sample analysis with a decline in GFR (40mL/min/1,73m2) and a previous history of hypothyroidism, hypertension and dyslipidemia. Further tests showed an urinary sediment with RBCs 20/HPF, dysmorphic RBCs (10%), proteinuria (278 mg/24 hours), positive antineutrophil cytoplasmic antibodies (ANCA)–myeloperoxidase (MPO), and antibodies against thyroid peroxidase. She was admitted in the Nephrology department of the hospital to perform a renal biopsy, which revealed a pauci-immune necrotizing glomerulonephritis with cellular crescents. She started treatment with methylprednisolone pulses for three days, oral prednisolone, 1mg/kg/day and cyclophosphamide. Due to the fact of hyponatremia and suspicion of cyclophosphamide cardiac toxicity, a switch was made to rituximab. She currently has a stable GFR of 37mL/min/1,73m2, without proteinuria and RBCs 7/HPF with 4% dysmorphic RBCs.

Conclusion: Although the presence of microscopic hematuria isn't by itself an indicator of a bad prognosis, a carefully collected clinical history, physical examination and other concurring laboratory findings are necessary to decide if additional studying is required. The case presented shows how microscopic hematuria lead to further investigation and testing, and finally, to a diagnosis of ANCA-associated vasculitis.

|CR13

VITAMIN B12 DEFICIENCY ANAEMIA WITH AN UNUSUAL PRESENTATION: THE IMPORTANCE OF A PROPER LABORATORY STUDY IN THE DIAGNOSIS

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Clinical report: 25-year-old female, black race, living in Portugal for six years. Personal history of chronic normocytic anaemia of unknown aetiology since 2020, requiring blood transfusions. Admitted to the emergency department in 2022 due to asthenia, anorexia, nausea/vomiting, which she stated having been present for five days. There were no complaints of fever, blood loss, neither other neurological/respiratory/gastrointestinal symptoms.

On physical examination, she presented with jaundice and pallor. Anaemia (haemoglobin 8.2g/dl, mean blood volume 98.8 fL, reticulocytes 2.2%) was documented on the hemogram. The peripheral blood smear showed marked anisopoikilocytosis with dacrocytes and schizocytes. Additionally, the blood study revealed increased lactate dehydrogenase (5097 U/L), bilirubin (direct 1.44 mg/dl /indirect 0.53 mg/dl) and decreased haptoglobin (< 1%), suggesting haemolytic anaemia.

Given her clinical condition and the laboratory findings, she was hospitalized for aetiological study, which revealed low vitamin B12 (124 pg/mL) and negative direct Coombs test, hemoglobin H test and osmotic fragility test. The red blood cells pathology study using the methodology HPLC (high-performance liquid chromatography) did not show any variant or relevant alteration. Other infectious and neoplastic causes were excluded.

Discussion: As the patient presented with vitamin B12 deficiency without any dietary restrictions, atrophic gastritis was investigated and confirmed by endoscopic gastric biopsy [positive anti-parietal cell antibodies (title 1/40) and low intrinsic factor (31 RU/ml)].

The pernicious anaemia hypothesis was supported by the analytical and clinical response to the administration of intramuscular vitamin B12 [improvement in reticulocytes (10.5%) and hemoglobin (9.9 g/dl) values and haptoglobin normalization (52 mg/dl)].

The presence of laboratory criteria for hemolysis, justified by ineffective hematopoiesis, guided the study towards screening for a hemolytic cause which, in the case of a young woman, included congenital or acquired pathology of the red blood cell.

Although there were no neurological symptoms or typical hematological changes, B12 deficiency anemia was confirmed, being relevant to the exposure of these clinical cases that can be associated with high morbidity.

|CR14

BLEEDING DIATHESIS AS THE INITIAL PRESENTATION OF ACUTE PROMYELOCYTIC LEUKEMIA

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A 59-year-old male with a medical history of hypertension, dyslipidemia, and chronic obstructive pulmonary disease, in the context of alpha 1 antitrypsin deficiency, was admitted at the hospital due to new onset of hemorrhagic oral blisters and spontaneous hematomas dispersed throughout the body.

A complete blood count was performed and showed severe thrombocytopenia (27.000), and mild leukopenia (1,0 x109/L). The comprehensive metabolic panel was innocent. He had no constitutional symptoms, and C-reactive protein was negative. Abdominal and renal ultrasounds showed no relevant changes and no other significant physical examination findings were observed. Viral studies were negative for Hepatitis B, C, HIV, and SARS-CoV-2. Urinalysis showed hematuria. The examination of the peripheral blood smear revealed 2 % blast cells. Coagulation studies showed normal activated partial thromboplastin time, elevated prothrombin time (15,5 seconds), and an international normalized ratio of 1,32. D-dimers were > 20 ug/mL, and fibrinogen was decreased (144 mg/dL).

Acute leukemia was suspected, and the patient was admitted to start all-trans retinoic acid treatment. Bone marrow aspiration showed massive infiltration of promyelocytes/blasts with abundant Auer rods. Further immunophenotypic screening showed the presence of 84% of myeloid blasts that had a heterogeneous expression of CD13 and were CD 33+, CD 34-, CD 38+, CD 64+, MPO+. This immunophenotype was compatible with acute promyelocytic leukemia (APL). Fluorescence in situ hybridization analysis further revealed the presence of a PML-RARA fusion and the karyotype showed the reciprocal 15;17 translocation which is characteristic of the APL and confirmed the diagnosis.

Acute promyelocytic leukemia represents a medical emergency with a high rate of early mortality. It is necessary to start ATRA treatment without delay, as soon as the diagnosis is suspected, to decrease the risk of complications associated with APL coagulopathy. It is paramount for the clinical pathologist reviewing CBCs results to perform a blood smear evaluation and thoroughly evaluate the presence/absence of immature granulocytes and rule out APL, especially, when bi/pancytopenia presents with bleeding diathesis.

|CR15

THE UTILITY OF SCATHERGRAMS TO IDENTIFIED PLASMODIUM IN SUSPECTED CASES - A RETROSPECTIVE STUDY

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Malaria is a disease caused by the Plasmodium parasite, which is transmitted through the bites of an infected female Anopheles mosquito. There are 5 different species of Plasmodium that affect humans: P. Falciparum, P. Vivax, P. Ovale, P. Malarie and P. Knowlesi. It is estimated that only in 2019, 229 million people were infected with malaria worldwide and of these, 409,000 died from the disease, mostly due to P. Falciparum, the most common species. P. Vivax, P. Ovale and P. Malarie can remain in the liver in a latent state. Early diagnosis and treatment reduce the burden of disease and mortality.

This work is based on a retrospective study, in which the results of the research of the Parasite Plasmodium are evaluated during 3 months in a central hospital.The study aimed to evaluate the utility of the graphics provided by Sysmex XN analyser to identify suspicious cases of infection by Plasmodium.We have analysed 74 research of Plasmodium and only 7 had the parasite. 5 of these had both rapid and peripheral blood smear (PBS) positive for Plasmodium Falciparum, 1 had only positive rapid test for Plasmodium Falciparum and one had both rapid test and PBS positive for Plasmodium Vivax. Only PBS of the Plasmodium Vivax had trophozoites and gametocytes. This is also the only one who had a different region, below neutrophils appearing in the scattergram. In this time being we also found 3 cases without any plasmodium search and suspicion that had a smaller but similar area appearing in the scattergram. These patients had another type of severe disease.

In conclusion we can see typical areas appearing for gametocytes in a WDF graphic of Sysmex XN. However, we cannot see trophozoites and we cannot also use this as a “screening exam” because we can find the same zone in other cases.

|CR16

CHANGE IN VIRAL RESPIRATORY INFECTIONS EPIDEMIOLOGY IN CHILDREN DURING THE COVID-19 PANDEMIC

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Viruses are the major cause of acute respiratory infections in children. Before the onset of the COVID-19 pandemic, in countries with temperate climate the epidemiology of these viruses exhibited a typical seasonal pattern, with influenza, coronavirus and respiratory syncytial virus infections peaking during the winter months, and adenovirus, bocavirus, metapneumovirus and rhinovirus detected throughout the year. However, several studies reported an altered epidemiology of respiratory virus infection during the pandemic, with low activity during the typical season and an interseasonal rise.

We retrospectively reviewed all laboratory results of viruses detected in respiratory specimens collected from children (0-18 years old) from 2018 to 2021, in a tertiary care Hospital in Portugal.

From 2018 to 2020, the results show a seasonal variation in viral infections, with peaks in the winter, between November and March – maximum number of cases 110 in January 2018, 101 in January and February 2019, 77 in January 2020. In the winter of 2020/21, the first winter after the pandemic, when preventive measures against COVID-19 were harsher, we observed an interruption of that pattern, with abnormally low numbers of infections (maximum 9 cases in January 2021. In contrast, during the summer of 2021, there was an unusual increase, coinciding with the relief of the restrictions when control of the infection by SARS-CoV-2 was better - 63 cases in July/August 2021 vs 1, 0 and 5 cases in 2018, 2019 and 2020, respectively.

Our work describes a disruption of the seasonal pattern of viral respiratory infections in children during the COVID-19 pandemic in Northern Portugal, with a virtual elimination during the usual peaking months, and an increase afterwards. This change in epidemiology is associated with the variation of non-pharmacological measures used for the mitigation of SARS-CoV-2, and provides evidence of their efficiency in the prevention of the transmission of respiratory infection.

|CR17

COMPARISON OF TWO METHODS FOR THE BINDING SITE'S FREE LIGHT CHAINS ASSAY: TURBIDIMETRY VS NEPHELOMETRY

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Introduction: Free light chains (FLCs) assays have become of the utmost importance in the diagnostic approach and follow-up of monoclonal gammopathies. Different analytical methods for measuring FLCs may return different values of its serum concentrations with substantial impact on patients' assessment. The Freelite® (The Binding Site, UK) assay is available on multiple platforms and it is the assay of choice in our center, paired with the BN™ II (Siemens, Germany) nephelometric system. However, a change to Freelite in the turbidimetric analyser Optilite® (The Binding Site, UK) is being considered.

Aim: To compare FLCs concentrations obtained with both methodologies and how they may affect patients' results.

Methods: 61 patients' serum samples were included in this retrospective study. Samples were tested on the BN™II and frozen up to one month before being tested with the Optilite® assay. The methods were compared by Bland-Altman Plot and Passing-Bablok Regression (x axis=BNII) in MedCalc (v14.8.1.0). Bias was calculated as mean ± standard error of the mean. Overall concordance between methods was assessed using semi-quantitative analysis for the FLCs ratio (3 samples were excluded for not having both FLCs measurements).

Results: Methods comparison for κFLCs showed Pearson's r = 0.949 with a slope of 0,8886 (95%CI 0,8433 to 0,9583), the bias was 3.9±1.3, the upper and lower limits of agreement were 22.7 and -15.0, respectively. For λFLC, Pearson's r = 0.997 with a slope of 1,0321 (95%CI 0.9958 to 1.0719). The bias was -0.1±0.4, the upper and lower limits of agreement the limits of agreement were 6.5 and -6.8, correspondingly. The overall concordance for the FLC ratio was 96.6%; with 5.3% being low, 72.5% being within reference intervals and 18.8% elevated. The 2 discordant cases (3.4%) were near cut-off points.

Conclusions: The concordance of the assay between the turbidimetric and the nephelometric methods appears satisfactory, although a few discrepancies could be evidenced. Correlation between methods was better for lambda than kappa FLCs. With the present study's testing conditions and samples, these assays were not entirely equivalent. Thus, the appropriate actions should be performed if a switch of methods is decided.

|CR18

EXTERNAL QUALITY ASSESSMENT: TRENDS AND DEVELOPMENTS

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What is the purpose of external quality assessment (EQA)? The definition is not harmonized. CLSI C48 defines as the "determination of the performance of laboratory tests through comparisons between laboratories."

In fact, what the EQA allows is to measure the laboratory (retrospective) bias from a consensus value (the target). It is essential to recognize that the true bias is clinical, i.e., the difference between the in vitro sample value and in vivo concentration. So, the control sample should replicate the human sample with a certain confidence, which is always a complex challenge given the variability and heterogeneity of these samples.

The confidence of the bias in quantitative results depends mainly on the homogeneity of the results (low variance) and their trueness (true biased). The use of consensus values based on subgroup results, e.g., shared testing, is emphasized. Whenever there is considerable heterogeneity of results, the probability of true bias is is not robust enough, statistically, and clinically.

On the other hand, if the results are qualitative, true/false, positive/negative, heterogeneity (due to false results) is not a critical limitation. It is because the EQA scheme provider determines the goal based on a true positive result.

Statistical methods used in EQA by interlaboratory comparison should follow the ISO 13528 standard. We suggest using the mean and standard deviation to measure the target in normal distribution data and measure homogeneity for quantitative results, respectively. The z-score expresses the position of a raw score in terms of its distance from the mean when measured in standard deviation. On the other hand, the error percentage represents the relative bias, typically a percentage. Measurement uncertainty allows measuring the randomness of the group or subgroup results. The heterogeneity of the exercise data strongly influences this measure so that it can be interpreted as complementary to the standard deviation. We also suggest consulting the EURACHEM/CITAC and the EFLM recommendations for EQA schemes.

On the other hand, the bias (false results) of qualitative data is measured through the agreement of the exercise results with the target. Performance grading can be done using the Misclassification Index Score (MIS), which ranks as a function of the number of discordant results in a series of exercises.

Posters

|P01

HISTIOCYTIC CELLS IN PERIPHERAL BLOOD SMEAR - AN ALERT FOR THE DIAGNOSIS OF INTRAVASCULAR LARGE B-CELL LYMPHOMA

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Intravascular large B-cell Lymphoma (IVLBCL) is a rare type of non-Hodgkin's lymphoma characterized by exclusive or predominant growth of neoplastic cells within the lumen of blood vessels, although they are rarely found in peripheral blood smears (PBS).

IVLBCL has a heterogeneous presentation and can be classified into variants, the classical variant, the cutaneous variant and the Hemophagocytic syndrome–associated variant. In this last one, nonneoplastic histiocytes can be observed in peripheral blood or bone marrow.

We present the case of a 37-year-old woman, with a six- month history of weight loss, fever and night sweats. She had anemia and thrombocytopenia, with a normal white blood cell count (WBC) and no morphological alterations in the PBS. The bone marrow aspirate was hypercellular, without evidence of myeloid or lymphoid disease and the immunophenotyping revealed no alterations. Genetic studies and extensive investigation of infectious, autoimmune or other neoplastic diseases were all negative. A diagnostic splenectomy was made which revealed only inflammatory reaction.

One month later, the patient maintained the same symptoms and was still anaemic, with a WBC of 19,6x103/µL (Neutrophils 7,94x103/µL, Lymphocytes 4,9x103/µL and monocytes 6,29x103/µL) and a normal platelet count. On the PBS, several monocytes, macrophages and some cells from the histiocytic lineage were observed and another bone marrow aspirate revealed hemophagocytosis. After this findings, peripheral blood and bone marrow aspirate were sent for immunophenotyping, again, which suggested a Large B-cell Lymphoma rich in T-cells and histiocytic cells. Hepatic biopsy was performed, revealing morphologic and phenotypic findings supporting the diagnosis of IVLBCL. According to the clinical presentation and laboratory findings, most likely the Hemophagocytic syndrome-associated variant.

When studying a patient with fever of undetermined origin, in which all other diagnosis have been excluded, or when histiocytic cells are observed in the PBS, this entity should be considered. This case is also important to remind us that the attentive observation of a blood smear can guide us to the diagnosis.

|P02

WBC SCATTERGRAM, A SUGGESTION OF UNSTABLE HEMOGLOBINS

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Introduction: Unstable haemoglobin variants shows decreased solubility and consequent intra-erythrocyte precipitation. Those variants are fairly rare and predispose to haemolytic events, either chronic or occasional. It has been described that this kind of haemoglobins interferes with peripheral white blood differential count, leading to abnormal and very peculiar scattergrams. These pattern can be a hint of those conditions.

Aim: To establish a relation between a distinctive scattergram pattern and the suggestion of unstable haemoglobin.

Case: JDM, male 58 years old diagnosed with unspecific familial haemaglobinopathy and chronic haemolysis. Splenectomy and frequent therapeutic phlebotomies with the following laboratory parameters:

Sysmex XN-1000: Red blood cells: haematocrit 55%, MCV 109.8fL, MCH 31.1pg, reticulocyte 34.15%; White blood cells: within reference values. WBC scattergram: abnormal/distinctive pattern with low fluorescent signal and low WBC differentiation.

Peripheral blood smear: RBC - Macrocytosis, presence of basophilic stippling and Howell-Jolly bodies; WBC - No significant findings.

Results: A blood sample was sent to a reference laboratory (INSA) for the study of haemaglobinopathies. The results of isoelectric focusing and HPLC suggested the presence of an unstable haemoglobin. The sample proceeded to genetic study with the following result: Heterozygoty for a mutation at HBB: c.295G>A p.(Val99Met) which gives rise to Hb-Koln.

Conclusion: Unstable haemoglobins have tendency to denature, precipitate and degrade leading to variable/silent electrophoretic and chromatographic patterns.

Most of the time, those patterns are underrated and the beginning of the haemoglobin variants study is delayed. A careful analysis of the scattergrams can be a clue to the presence of this condition.

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|P03

IS ARTERIAL BLOOD GAS HEMOGLOBIN TRUSTABLE, ESPECIALLY WHEN ITS VALUE DROPS HARD?

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Introduction: Arterial Blood Gas (ABG) analysis is a fast, low-quantity-sample-requiring procedure, considered a “Point of Care” (POC) test. One of its relevant parameters is Hemoglobin (Hb), which is very important in Emergency and Intensive Care Units (EU and ICU), especially in patients needing blood transfusion.

Objectives: The aim of this study is to verify if there is a correlation between the values of Hb measured in an ABG sample and in hemogram, which is the reference method, in patients needing blood transfusion therapy (Hb≤7 g/dL) and in patients with no critical values (Hb>7g/dL).

Methods: Retrospective observational study, between October 2020 and February 2021, including paired results of Hb measured in ABG (syringe safePICO® Aspirator, Radiometer®) and hemogram (S-Monovette® K3 EDTA, Sarstedt®) samples

delivered to the Emergency Laboratory at the same time, from EU and ICU. Two groups of results were analysed based on the value of Hb obtained in the hemogram, one with Hb≤7 g/dL (Group 1) and another with Hb >7 g/dL (Group 2). The analysers used to measure the Hb were DxH 900® Hematology Analyzer, Beckman Coulter®, for hemograms and ABL800® FLEX blood gas analyzer, Radiometer®, for ABG. All statistical analyses were performed using the Excel® software, by applying a regression analysis to compare the Hb values measured in ABG and in hemogram.

Results: This study included 1685 pairs of Hb results, 89 belonging to Group 1 and 1596 to Group 2. In Group 1, the mean of Hb values in the hemogram and ABG was 6,469 (2,4 - 7) g/dL and 6,639 (2,1 - 9,7) g/dL and Standard Deviation (SD) was 0,919 and 2,546, respectively. The Regression Analysis showed a Pearson's Correlation Coefficient (R) of 0,755; a Coefficient of Determination (R2) of 0,571 and a ρ-value of 8,24E-9. In Group 2, the mean of Hb values in the hemogram and ABG was 10,276 (7,1 - 19,6) g/dL and 10,524 (5,9 - 20,3) g/dL and SD was 0,778 and 2,970, respectively. The Regression Analysis showed R=0,958; R2=0,918 and ρ-value=7,13E-29.

Conclusion: This study demonstrates that there was a strong correlation between Hb values obtained in ABG analysis and in hemogram. However, it was stronger with Hb>7 g/dL in hemogram. That means that values of Hb in ABG measured in ABL800® FLEX blood gas analyzer can be trusted, even to determine transfusion therapy.

Disclosure - No conflicts of interest.

|P04

ANAEMIA OBSCURED BY SEVERE HYPERTRIGLYCERIDEMIA

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Introduction: Severe hypertriglyceridemia (HTG), defined as a fasting triglyceride level ≥ 500 mg/dL, has been associated with various complications, such as increased cardiovascular risk or recurrent acute pancreatitis. HTG results from increased plasma concentration of lipoproteins responsible for the transport of triglycerides (TG). Severe elevation of TG levels can be caused by rare monogenic autosomal recessive disorders. The turbidity of a highly lipemic serum or plasma sample may interfere with various laboratory methods, one of which is colorimetry. As haemoglobin (Hb) concentration is determined by spectrophotometry, an elevated TG level can interfere with the correct measurement of Hb levels and may even mask anaemia.

Clinical Case Report: A 44-year-old, male patient with a history of hypertriglyceridemia and various episodes of acute pancreatitis was evaluated because of severely elevated TG levels and symptoms compatible with acute pancreatitis. He had a positive family history – a sibling with hypercholesterolemia and hypertriglyceridemia – however genetic testing for familial HTG was inconclusive. Blood samples were collected into anticoagulated and gel separation tubes. After centrifugation, the patient´s serum had a milky appearance, TG level was 6743 mg/dL. The EDTA treated plasma sample was analysed using a Sysmex® XE-5000 analyser and showed a Hb level of 13.9 g/dL (mean corpuscular haemoglobin (MCH) level was 31.4 pg and mean corpuscular haemoglobin concentration (MCHC) was 37.5 g/dL). The sample was warmed and reanalysed presenting a Hb level of 14.4 g/dL (MCH 31.9 pg, MCHC 38.5 g/dL). When the turbidity of the sample became apparent, the sample was processed once again using the same analyser through the fluorescent channel giving a calculated optical Hb (HGB-O) level of 11.5 g/dL, which allowed for the correction of the remaining indices too – MCH 25.5 pg and MCHC 30.7 g/dL.

Conclusion: In the presence of factors that cause turbidity (for example marked lipemia, hyperbilirubinemia or marked leucocytosis), Hb concentration must be measured through the fluorescent channel and dependent parameters (MCH and MCHC) should be corrected. The turbidity of the sample might cause a falsely elevated Hb level that in turn might obscure anaemia.

|P05

ONE YEAR OF COVID-19 IN PEDIATRIC AGE GROUP AT CENTRO HOSPITALAR UNIVERSITÁRIO DE SÃO JOÃO

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Background: The symptoms of COVID-19 in children are similar to adults but appear to be milder. The most common clinical findings are fever or chills and cough, and close to one-third of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections are asymptomatic. However, in rare cases, children can be severely affected, exhibiting an hyperinflammatory syndrome similar to incomplete Kawasaki disease or toxic shock syndrome - pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS), and requiring hospitalization.

Objective: The present work aimed to access the population of children infected with SARS-CoV-2 admitted to a COVID-19 front-line hospital in northern Portugal.

Materials/methods: An observational retrospective analysis was performed. All children and adolescents from 0 to 18 years old that were admitted to the hospital between March 1 2020 and March 1 2021 and tested for the presence of SARS-CoV-2 viral RNA were included. Data was collected and reviewed from hospital records: SClinicoV2, Alert, jONE and ClinidataXXI.

Results: Among a total of 14179 children that were submitted to a SARS-CoV-2 RT-qPCR test, 684 had a positive result (4,82%). This value represents 8,4% of the total number of COVID-19 infections identified at the same time. Among them, 332 were female (48,5%) and 352 were male (51,5%). Eighty-two (12,0%) of these children were younger than 6 months, 130 (19,0%) between 6 month-1 year, 46 (6,7%) between 1-3 years, 63 (9,2%) between 3-6 years, 80 (11,7%) between 6-10 years, 124 (18,1%) between 10-14 years and 159 (23,2%) between 14-18 years. Only 1,5 % exhibit PIMS-TS.

Conclusion: Our results are in line with the available data published. According to American Academy of Pediatrics, the pediatric COVID-19 infection represents 13.5% of the total number of cases and the PIMS incidence, although still uncertain, appears to occur in less than 1% of children.

|P06

RESULT OF THE PORTUGUESE PILOT EQA PROGRAM IN SARS-COV-2, PCR

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Introduction: The Portuguese National External Quality Assessment Program (PNAEQ) with the National Reference Laboratory (NRL) organized the 1st External Quality Assessment pilot program (EQA) for detection of SARS-CoV-2 virus by molecular methods.

Objective: Implementation of an external quality assessment program for molecular detection of SARS-CoV-2 virus, for performance evaluation and monitoring of implemented tests in Portuguese Laboratory Network, as well as the differentiation between the new coronavirus and the seasonal coronavirus.

Methods: For this EQA pilot, 4 control samples (30µL) containing extracted nucleic acids were prepared by NRL from pools of extracted nucleic acids from positive SARS-CoV-2 and seasonal coronavirus samples. The samples were tested for homogeneity and stability studies and selected the intended concentrations according with the Cycle Threshold (CT).

Two of the samples were negative, one contained SARS-CoV-2 and the other contained seasonal coronavirus (hCoV HKU1).

The results (reported in REDCap) were analysed, comparing the qualitative results (interpretations) of each laboratory, with the expected results determined by NRL at INSA. Preliminary, global and individual reports were issued.

Results: Samples were stable and suitable according stability analysis. The program accounted with 25 laboratories. Regarding extra analytical questions: 13 perform biologic product collection for the detection of SARS-CoV-2, mainly in the upper respiratory tract; all mentioned the use of the recommended IPE; 20 receive samples from collection points and/or from other laboratories and implemented safety rules for the handling and transport of the samples.

For the two negative samples we obtained 100% of correct results (coronavírus not detected). It was reported a false negative for both SARS-CoV-2 and seasonal coronavirus (hCoV HKU1).

Conclusion: The evaluation of the extra-analytical questions showed that the laboratories complied with the national guidelines. Generally, the analytical performance was good. The two false negative reported might suggest the need to verify the sensitivity of the implemented methods. Only one laboratory performed the differential diagnosis for the identification of seasonal coronavirus.

Participation in EQA programs, give a reliable estimate of the assays performance required for patient care, also contributing to the harmonization of the implemented methods and to the improvement of the analytical quality.

|P07

RARE FORM OF ACUTE HEPATITIS DUE TO HERPES SIMPLEX 2 VIRUS INFECTION AFTER KIDNEY TRANSPLANTATION

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Introduction: Herpes simplex virus 2 (HSV-2) is a rare etiologic agent of acute hepatitis. This condition can progress to acute liver failure and is associated with a high mortality rate when untreated. Immunosuppression and pregnancy are known risk factors. We present a case of HSV-2 acute hepatitis following a kidney transplantation.

Clinical Case: A 49-year-old male, with history of HSV-2 genital infection, hypertension, cerebrovascular disease, and chronic kidney disease, received a kidney transplant. The surgery was successful, but a week later liver function tests became abnormal. Upper levels were reached within five days (aspartate transaminase 143 U/L, alanine transaminase 639 U/L, γ-glutamyltransferase 318 U/L); no signs or symptoms were documented. Abdominal ultrasound solely reported lithiasic steatopathy. Differential diagnosis included drug related and viral etiology hepatitis. Hepatotoxic drugs were promptly suspended or dose adjusted, without any clinical improvement. Blood molecular diagnosis (RT-qPCR) returned positive for HSV-2. Unfortunately, viral load was not determined. HSV-2 acute hepatitis was assumed and acyclovir was initiated, with posterior decrease of liver function tests. After three days of treatment, repeated RT-qPCR returned negative for HSV-2. The patient remained asymptomatic and recovered hassle free. Serologic screening prior to transplant was positive for HSV-2 (immunoglobulin G 42 RU/mL), and immunoglobulin G titers remained stable after four months of follow-up; immunoglobulin M was always negative.

Discussion: Early diagnosis of HSV-2 hepatitis may be challenging because of uncharacteristic signs and symptoms. RT-qPCR was fundamental to confirm the diagnosis and initiate appropriate therapy. Immunoglobulin titers were helpful in defining

pre-transplant risk stratification and prevention. In this case of previous known HSV-2 infection, prompt antiviral prophylaxis was not prescribed. Available bibliography supports such protocol. Therefore, clinicians should consider HSV-2 prophylaxis in all patients prior to kidney transplant. Taking previous history into consideration, we believe this case shows an acute hepatitis, in an immunosuppressed patient, caused by HSV-2 reactivation; facilitated by the lack of antiviral prophylaxis.

|P08

ROBUST AND AGE DEPENDENT IMMUNOLOGIC RESPONSE OF HEALTHCARE PROFESSIONALS VACCINATED WITH THE PFIZER-BIONTECH COVID-19 VACCINE

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Objectives: To evaluate the immunologic response of healthcare professionals (HCPs) to COVID-19 induced by the Pfizer-BioNTech vaccine (tozinameran) at CHTS.

Materials & Methods: The serum levels of IgG antibodies against the receptor binding domain of the S1 subunit of the spike protein of SARS-CoV-2were determined using the SARS-CoV2 IgG II Quant kit (Abbott) at two time points: T0, 0-2 days after the first vaccine dose; and T1, 30-33 days after first dose(i.e. 9-12 days after second dose). The cutoff value was 50.0 AU/mL. At T1, positive results were stratified in 3 high titer probability curves: 51-2999, probability <90%; 3000-6299, 90-95% probability; >6300, probability >99%.

Results: A total 1394 HCPs were included in the study: average age, 41.9±10.9 years; 78.8% female; 98.9% with 2 vaccine doses. T0 values were available for 645 HCPs: 82.8% negative, 17.2% positive. At T1, serologic levels were distributed as follows: <50, 0.1%; 51-2999, 1,9%; 3000-6299, 2,9%;>6300, 95,1%. There were no significant differences for T1 values vs. gender, p=1.000 (Fisher exact test), but there were significant differences vs. age groups <30, 30-40, 40-50, and >50, p=.030, and a statistically significant difference between <30 and >50 age groups, p=.002<.008. There was a significant difference at T1 levels vs. number of doses, p<.001: 2 doses, 95.9% assigned to >6300; 1 dose, 86.6% assigned to intermediate levels (51-6299), and only 13.3% to >6300. This difference was also statistically significant for HCP positive at T0, p<.001: 2 doses, 100.0% assigned to >6300; 1 dose, 92.3% assigned to intermediate levels, 7.7% to >6300. For those HCP positive at T0 there were no significant differences at T1 levels after 2 doses, compared to those negative at T0 (p=.349).

Conclusions: Our data demonstrates a robust immunologic response to Pfizer-BioNTech COVID-19 vaccines: gender independent, stronger below the age of 30, and requiring 2 doses, independently of previous exposure to SARS-COV-2. Our data also demonstrates the utility of serologic tests to assess the quality of immune response, which, eventually, may provide important insights for the optimization of COVID-19 vaccination guidelines. This is an ongoing study: data at 6 and 12 months will clarify the durability of the immune response to vaccine.

|P09

URINARY FREE CORTISOL MEASUREMENT: COMPARISON OF TWO AUTOMATED IMMUNOASSAYS

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Introdution: Cortisol is a steroid hormone synthesized in the adrenal cortex. Most of serum cortisol circulates bound to protein but only the free cortisol is biologically active. Free cortisol (FC) is excreted unchanged in the urine (UFC) and correlates well with serum cortisol. Immunoassays (IA) are used to evaluate UFC but the presence of conjugated cortisol

metabolites that can cross-react with IA antibodies led to the development of IA that required an extraction of FC prior to analysis. Recently, IA with increased specificity to FC have been developed to surpass the cross-reactivity.

Objective: Evaluate the performance of two IA to determine the UFC concentration in 24 hours (24UFC) samples, with and without the extraction step.

Methods and Materials: During one month, 37 samples for 24UCF (11 men and 26 women) were processed. The 24UCF were assayed in the Cobas® e411 (Roche®) analyzer (CR/IA), with a preceding extraction step with dichloromethane, and in the Alinity i® (Abbott®) analyzer (AA/IA), a fully automated onestep IA without extraction, both according to the manufactures instructions. Manufactures reference values (RV) are: 36 – 137 µg/24h for the CR/IA and 4,3 – 176 µg/24h for the AA/IA. The results were evaluated using the Pearson correlation.

Results: The comparison of the results showed a positive high correlation between the two IA (y= 0.6357 + 0.9114; r2=0.949). Mean UFC were 5.80 ± 9.65 µg/dL in the CR/IA and 4.60 ± 6.30 µg/dL in the AA/IA. Considering RV, CR/IA revealed 6 samples above range and AA/IA 3 samples, exposing 8% of discrepant clinical decision. In 16 samples (43%) bias% between both methods were above 20%.

Conclusion: The UFC concentration is crucial to the diagnosis of hypercortisolism and so, faster results are important to clinical decision. The results of the two IA correlated very well with low impact on clinical decision. Direct IA without extraction seems suitable to a faster laboratory response.

|P10

DIAGNOSTIC UTILITY OF THE IMMUNOCAP TEST FOR ANTI-PIGEON IGG IN BIRD-RELATED HYPERSENSITIVITY PNEUMONITIS

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Introduction: Bird fancier's lung is a form of hypersensitivity pneumonitis (HP) caused by an immune reaction to inhalation of bird derived antigens. Its diagnosis is based on a history of exposure to birds or their products and serological, radiological, and pathological findings. Antigen avoidance is advised when bird-related HP is suspected; hence, the importance of identifying causative antigens, with pigeons being the most frequently reported among avian sources. In our lab, anti-pigeon specific IgG is determined by a fluoroimmunoenzymatic assay (ImmunoCAP, Thermo Fisher).

Objective: To evaluate the diagnostic performance of anti-pigeon IgG determinations in our lab, namely, the ability to discriminate patients diagnosed with HP from patients with other lung diseases.

Methods: Past determinations of IgG antibodies in the serum of 57 HP patients and 108 patients with other lung diseases (mainly COPD, asthma, idiopathic pulmonary fibrosis, other interstitial lung diseases) were subjected to t-test and ROC analyses (Excel Stat Plus). Sensitivity, specificity, predictive values, accuracy, odds ratio and Youden's index were determined.

Results: Patients were 62±15 years of age, 54% male, and pigeon exposure was referred by 37%, mostly among the HP group (56% vs. 30%). Anti-pigeon IgG concentrations were 237±425 and 14±28 mg/L in the HP and other disease groups, respectively (p<0.000). The area under the curve was 0,86 (95% CI 0,79-0,93). Considering the cut-off currently applied (17mg/L), for concentrations above 16,5mg/L, the following results were found: sensitivity 75% (95% CI 64-87%), specificity 84% (95% CI 77-91%); positive and negative predictive values 72% and 87%, respectively; accuracy 81% and odds ratio 16,4. A higher Youden's index (0,65 vs. 0,60) was obtained using 22mg/L as threshold, associated with higher specificity 94% (95% CI 90-99%), accuracy (86%), and odds ratio (40), at the expense of lower sensitivity (70%; 95% CI 58-82%).

Conclusions: Few publications have focused on the usefulness of ImmunoCAP anti-pigeon IgG test in the diagnosis of bird-related HP. This study demonstrated good performance of this quantitative method, its high specificity above 22mg/L and special value, along with other suggestive findings, for the confirmation of suspected cases.

|P11

DELTA BETA THALASSEMIA: LEARNING FROM EXTERNAL QUALITY ASSESSMENT

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Introduction: The Portuguese National External Quality Assessment Program (PNAEQ), in 2018 sent a sample simulating a case of Delta beta (δβ) thalassemia in the haemoglobinopathies program, in order to evaluate the participant's performance.δβ thalassemia results of a deletion in the genes delta and beta of chromosome 11. Although its definitive identification requires a genetic analysis, the hematologic evaluation allows its presumptive identification.

The hematologic phenotype of hetherozigotics for δβ-thalassemia is identical of the β-thalassemia minor, with microcytosis and hypochromia, but the percentage of HbA2 is not increased and the Hb F is usually high (5 -20%).

Objective: Evaluate the PNAEQ participant's performance for determining HbA2, HbF, and result's interpretation, in a sample simulating δβ-thalassemia carrier.

Methods: PNAEQ organizes, in collaboration with an expert work group, three rounds/year, with control and real patient samples, and case-studies for the evaluation of haemoglobinopathies.

The sample was prepared from whole blood and umbilical cord blood in order to simulate normal HbA2 and increased HbF, and sent on the 1st round of 2018. The participant's results were statistically evaluated for HbA2 and HbF. The results for fraction identification and result interpretation were evaluated according to the work group results.

Results and discussion: the sample simulated a 13-year-old girl with hypochromic microcytic anaemia, with excluded iron deficiency and deletional alfa-thalassemia, wasdistributed to 17 participants (percentage of participation 88,2%).

On the fraction identification, 2 laboratories didn't identify HbF and 3 didn't identify HbA and 1 didn't identify HbA2. The mean quantification of HbA2 was 2,2% (normal), (min= 2,0 and max=3,8) and for HbF was 16,2% (incresed) (min= 11,5 and max=36,9)

Only 5/15 laboratories chose the interpretation as carrier of δβ-thalassemia. It is essential to perform the presumptive identification of dβ-thalassemia carrier in order to clarify the hypochromic microcytic anaemia, as well as recommend the partner study in adult life, to identify possible couple risk and prevent severe cases of haemoglobinopathies.

Conclusion: The results indicate that it is necessaryto continue the process of performance evaluation and continuous training in this area, aiming for the continuous improvement of results and further clinical evaluation.

|P12

DELTA THALASSAEMIA IDENTIFIED BY A DECREASE IN HAEMOGLOBIN A2 IN THE HAEMOGLOBIN A1C TEST

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Introduction: Thalassaemia results from unbalanced haemoglobin synthesis caused by decreased production of at least one polypeptide chain of beta, alpha, gamma or delta globulins. The measurement of haemoglobin A1c by HPLC (High Pressure Liquid Chromatography) allows the detection and quantification of abnormal haemoglobin chains leading to the suspicion of haemoglobinopathies.

Objectives: Identify the decrease of Hemoglobin A2 expression and subsequent characterization by hemoglobin electrophoresis after the measurement of an HbA1C sample.

Population and methods: We present a case of a 60-year-old man from a haematology appointment with a request for haemoglobin electrophoresis after determination of glycated haemoglobin test. A HPLC technique was used to determine HbA1c using the HA-8180T equipment from A.Menarini diagnostics. The haemoglobin electrophoresis was performed in the Minicap-Flex equipment from Sebia by capillary electrophoresis.

Results: when studying the HbA1c a result of haemoglobin A2 of 1.4% below the normal value (reference value 1.5-3.5%) was detected. The patient had a mild anaemia with haemoglobin of 12.4 g/dL (reference value 13.5-17.0 g/dL) with normal erythrocyte indices. Haemoglobin electrophoresis detected haemoglobin A2 with a value of 1.1% and the presence of another peak with a different migration and whose value was 0.9%. This was identified as a variant of the haemoglobin A2 delta chain.

Discussion of results: Quantitative changes in haemoglobin A2 should be a warning signal when obtained from one haemoglobin A1c test. Decreased A2 values are infrequent in contrast to the increase in A2 that is associated with beta thalassaemia. Detecting the presence of a decrease in haemoglobin A2 production attributed to a variant of the delta chain, allowed the detection of delta thalassaemia, using haemoglobin electrophoresis conjugated to normal erythrocyte indices.

|P13

TRISOMY 18 IN LARGE B CELL LYMPHOMA

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Introduction: Diffuse large B cell lymphoma (DLBCL) is the most frequent lymphoma, within non-Hodgkin lymphomas, representing 25 to 40% of the latter. DLBCL has three main morphological variants: centroblastic, immunoblastic and anaplastic. The centroblastic type is the most frequent and is most common genetic alterations, are the translocation t(14;18), involving the BCL2 gene, that occurs in 35% of cases and the loss of the ING tumour suppressor gene, which represents 30% of cases.

Clinical Case: Female patient, 36 years old, diagnosed with DLBCL on excisional biopsy of right submaxillary gland.

According to the anatomopathological study, it presented a centroblastic phenotype, with a proliferative index (ki67) of 60%. The neoplasm had CD20+, CD3-, CD10+, BCL6+, MUM1+, BCL2+, MYC-, CD5-, CCND1-, CD21+, EBER-, CD23-. However, it did not show structural changes in the BCL6, MYC, IRF4/DUSP22, IGH genes.

The study also highlighted negative viral serologies and bone marrow examination without changes.

Our lab received a piece of the biopsy for flow cytometry immunophenotyping, which revealed no abnormal population. The same sample was processed for Fluorescent in Situ Hybridization (FISH) study t(14;18), using Metasystems probes (IGH/BCL2). Our findings suggested trisomy 18, and to exclude the possibility of structural changes of the BCL-2 gene, we used BCL2 break apart probe, which showed no alterations and confirmed our initial findings.

Discussion: Trisomy 18 is normally associated with complex cytogenetic modifications, not being usually the primary karotypic change. The gain of chromosome 18 could be responsible for the BCL-2 expression, observed in this case. BCL-2 (BCL2 Apoptosis Regulator) is a protein-coding gene and is usually associated with Follicular Lymphoma and High Grade B-Cell Lymphoma with MYC and/or BCL2 and/or BCL6 rearrangement.

Our findings reinforce that FISH is a useful and necessary tool on clarifying certain genetic alterations in lymphoma diagnosis, aiding clinicians in the diagnosis and treatment of this pathologies.

|P14

ACUTE PROMYELOCYTIC LEUKEMIA: A MEDICAL EMERGENCY

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Introduction: Acute promyelocytic leukemia (APML) is a subtype of acute myeloid leukemia (AML) involving the fusion of the retinoic acid receptor alpha (RARA) gene at 17q21.2 with the promyelocytic leukemia (PML) gene at 15q24.1, although additional secondary cytogenetic changes have been described. Treatment with all-trans retinoic acid (ATRA) and arsenic trioxide can lead to complete remission in most cases. Unfortunately, early mortality is high because of serious accompanying coagulopathy. Quick diagnosis and treatment initiation is of paramount importance.

Clinical Case Report: A 21-year-old female patient with unremarkable past medical history was brought to the emergency department after being found unconscious and unresponsive. On admission, the Glasgow Coma Scale was 6, requiring intubation and ventilation. Physical examination described bruising of the lower extremities. A family member reported a 3-day history of myalgia associated with subfebrile temperatures. Imaging revealed extensive cerebral haemorrhage. The lab results showed haemoglobin 9.8 g/dL, leukocytes 159.25 x 109/L, neutrophils 0.8 x 109/L, blasts 146.51 x 109/L, platelets 39 x 109/L, lactate dehydrogenase 802 U/L, partial thromboplastin time 28.9 s, prothrombin time 16.1 s, prothrombin-proconvertin time 0.79 U/mL, fibrinogen 161 ng/dL and D-dimer 1.21µg/ml. The peripheral blood smear presented rare abnormal promyelocytes and leukoblasts with numerous Auer bodies, suggestive of the microgranular variant of APML. To aid in rapid diagnosis and treatment initiation, fluorescent in situ hybridization (FISH) was performed and detected the t (15; 17) (q24; q21) translocation in 86% of analysed cells, prompting treatment with ATRA. Immunophenotyping identified 97% of myeloid blasts, the phenotype being compatible with AML but also the presence of blasts with some expression of CD34 and HLA-DR that are normally negative in APML. Karyotyping showed translocation between the long arms of chromosomes 15 and 17. PML-RARA bcr3 fusion transcript was detected by molecular genetics.

Conclusion: The correct evaluation of the peripheral smear allowed emergent reporting of the suspected haematological emergency. The FISH method was essential in the initiation of the correct targeted treatment of this specific PML-RARA mutation.

|P15

THE IMPORTANCE OF THE PRE-PRE-ANALYTICAL PHASE IN COAGULATION: IN REVISION TWO CLINICAL CASES

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Introduction: There are only a few studies related to the pre-analytical phase, in other words, to the selection of tests performed by prescribing clinicians. It is thought that the percentage of errors made at this stage may be greater than the sum of all the other errors committed in the pre-analytical, analytical and post-analytic phases, probably due to the limitations of clinicians' laboratory knowledge and the complexity of the coagulation tests, particularly in the therapeutic interferences.

Objective: Expose two situations, where both patients where under treatment with direct oral anticoagulant therapy (DOAC), in which the determination of Factor V was required for the diagnosis of liver failure, following an extended Prothrombin Time (PT). At the same time, we intend to highlight the importance of the Clinical Pathologist (CP) capability of validating results, who may or may not add other laboratory tests for the interpretation and consolidation of the results.

Discussion: Anticoagulant therapy with therapeutic doses of DOAC is nowadays frequently used and does not require monitoring or causes significant changes in routine tests (TP / APTT). However, it is known that these drugs do interfere with the ´´in vitro´´ coagulation mechanisms, and these results are not correlated with the ´´in vivo´´ mechanisms, as the levels of coagulation factors are lower than expected, and higher in other coagulation proteins, which can eventually lead to misdiagnosis.

Conclusion: The clinical information provided by the clinicians is fundamental for the validation of the results provided by the CP. As so, it is essential to include in the clinical information provided by clinicians the anticoagulant therapy that is being used, and it is the CP responsibility to communicate and elucidate the clinician of all the steps that led to the result being rejected or accepted.

|P16

KINETICS OF THE SEROLOGIC RESPONSE GENERATED BY SARS-COV-2 INFECTION

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Introduction: In December 2019, a new type of coronavirus (SARS-CoV-2, responsible for an Acute Respiratory Distress Syndrome – COVID-19), was identified for the first time in Wuhan, China. New tests against were developed early in 2020.

Objective: Kinetics of the serologic response generated by SARS-CoV-2 infection.

Methodology: Antibodies (Ab) tested: ECLIA anti-nucleocapsid total (NTot) Ab (Elecsys anti-SARS-CoV-2 Cobas ® 8000) and CLIA anti-nucleocapsid IgG (NIgG) Ab (SARS-CoV-2 IgG Architect i1000) and anti-spike IgM (SIgM) Ab (SARS-CoV-2 IgM Architect i1000).

100 sequential samples collected before November 2019 were used to study the specificity of tests (CI 95%). Samples from immunosuppressed or undergoing hemodialysis or chemotherapy patients were ruled out.

492 samples from 127 patients with Sars-CoV-2 infection confirmed by RT-PCR were taken at 3-day intervals, from the onset of symptoms to 23 days after, to evaluate the serological response to infection.

Seroconversion rate for each 3-day interval per test, first test to become positive per patient and the number of days between seroconversion of the first test and the seroconversion of the remaining tests per patient were evaluated.

Results: Specificity: NTot 100% (CI 94,4-100); NIgG 99% (CI 94,6-99,9); SIgM 98% (CI 93,0-99,8).

Among the 127 SARS-CoV-2 infected patients, we can report that:

a) Seroconversion rate was slightly higher for SIgM;

b) 50/127 patients were either already positive in the first 3-day interval or did not seroconvert;

c) SIgM seroconverted first in 30/77 followed by 17/77 where all 3 tests seroconverted simultaneously ($p<0.05$);

d) In remaining cases, first test to be positive was NTot, NIgG or a combination of two of the three tests;

e) In 23 of the 37 patients in who it was possible to track the process since seroconversion of first test to seroconversion of remaining tests, the gap never exceed one 3-day interval.

Conclusion: All tests present good specificity.

SIgM became positive earlier in more patients but all tests became positive up to 3 days in most of the patients.

There is no significant difference between NTot and NIgG tests by seroconversion rate and by first test to become positive per patient.

|P17

EVALUATION OF THE PANBIOTM COVID-19 ANTIGEN RAPID TEST (ABBOTT)

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Objective: Evaluation of the Abbott PanbioTMCOVID-19 antigen (Ag) rapid test (RT) in screening and diagnose of SARS-CoV-2 infection in adult subjects seeking the Emergency Room (ER) at Centro Hospitalar Universitário de São João (CHUSJ), Porto, Portugal.

Materials/Methods: The diagnostic value of the Panbio™ was determined in comparison to RT-qPCR reference method in subjects aged 18 and over suspicious of SARS-CoV-2 infection that were admitted in our ER. Participants were asked about the

onset of symptoms and risk of exposure to SARS-CoV-2. They were sampled for routine RT-qPCR testing, using a combined nasopharyngeal/throat swab and a concurrently nasopharyngeal swab for Panbio™. All samples were analyzed within a maximum of an hour. This study was approved by the medical research ethics committee of CHUSJ and all participants gave their fully oral informed consent.

Results: 186 subjects were enrolled. Collected samples for the Panbio™ were processed in a level 2 biosafety cabinet and results were recorded after 15 minutes of assay initiation, by two independent observers (blinded to each other and to the PCR results). Specificity and sensibility, as well as, positive and negative predictive values of the Panbio™ were calculated using the RT-qPCR results as reference test. Most participants were female (101 vs85) and despite gender most were between 20 and 55 years old. Nearly, all individuals reported symptoms, most frequently myalgia, sore throat, migraine and altered sense of smell or taste. Duration of symptoms varied between 1-5 days and 29% reported prior positive SARS-CoV-2 contact. Specificity of the Panbio™ was 100% while sensitivity was 86.5%. There were no false positives. False negative results were observed in subjects with high RT-qPCR Ct-values reflecting low viral load levels in nasopharyngeal samples. Restricting RT-qPCR test positivity to Ct-values under 30, yielded test sensitivities of 94.3%. Despite Ct-values, the positive predictive value was 100% while the negative predictive value ranged was 90.7% or 96.6%, the later using Ct-values under 30.

Conclusion: Panbio™ allows fast screening of putative COVID-19 patients. Individuals with positive results can be rapidly isolated but those who test negatively and experience COVID-like symptoms should be further tested by RT-PCR.

|P18

ELIZABETHKINGIA SPP. PULMONARY CO-INFECTION IN SEVERE COVID-19 PATIENT

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Introduction: Elizabethkingia spp. are aerobic, Gram-negative bacilli (GNB). Recently, they emerged as opportunistic nosocomial pathogens responsible for life-threatening infections in severely ill patients previously treated with large-spectrum antibiotics (ATB). No recommendations for empirical treatment or clinical breakpoints (BP) are yet available. Intrinsic resistance to generally recommended ATB for treatment of GNB infections have been described; therapeutic options include fluoroquinolones, Trimethoprim-Sulfamethoxazole (TMP/SMX), or Piperacillin-Tazobactam (Pip/Taz).

Clinical Case: We present the case of a 42-year-old man infected with SARS-CoV-2 and admitted to an Intensive Care Unit for cardiogenic shock, requiring invasive mechanical ventilation and Extracorporeal Membrane Oxygenation. 33 days into admission, after several cycles of ATB to treat ventilator-associated pneumonia, he presented fever and purulent sputum, with increased inflammatory parameters. Blood, urine, and respiratory samples were collected for microbiological testing; empirical therapy with Meropenem and Vancomycin was started. Sputum Gram staining showed GNB; the specimen was inoculated in blood, chocolate, and MacConkey agar; mucoid, non-fermenting colonies grew after incubation at 37ºC, and were identified by Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry as Elizabethkingia miricola. The antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disc diffusion method and interpreted with EUCAST non-species-related PK/PD BP. The isolate was sensitive to Ciprofloxacin (CIP) and resistant to Amikacin, Cefepime, Ceftazidime, Imipenem, Tobramycin, and Pip/Taz. A MIC of 0,5µg/mL for TMP/SMX was found, but no BP are available. ATB therapy was switched for CIP, with favorable clinical evolution.

Discussion: In ICU, where high selective antimicrobial pressure occurs, less common GNB like Elizabethkingia spp. should be considered a differential diagnosis of nosocomial infections, especially in COVID-19 patients under heavy corticotherapy. Being a multidrug-resistant pathogen, the microbiology laboratory plays a pivotal role, ensuring an appropriate AST is correctly performed and reported to the clinical team promptly.

|P19

AGGREGATIBACTER ACTINOMYCETEMCOMITANS ENDOCARDITIS – CASE REPORT

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Introduction: Aggregatibacter actinomycetemcomitans is a Gram-negative slow growing bacillus and a member of the HACEK group, usually found as part of the oral commensal bacteria. It is able to cause periodontitis and, rarely, extra-oral infections secondary to hematogenous dissemination. Bacterial endocarditis is the most common infection outside the oral cavity.

Case presentation: A 57 year-old man went to the emergency room with a history of daily feverish peaks, accompanied by weight loss of 3 kg, lasting for more than 30 days. No other symptoms were noted. Mitral prolapse is of relevance in his past medical history.

Upon examination, he was febrile (38.,9ºC) but maintained hemodynamic stability. Oral examination revealed fragmented teeth but no apparent cavities.

Blood testes showed leucocytosis (white blood count 16,2x10g/L) with neutrofilia, hypochromic microcytic anaemia (hemoglobin 9,8g/L) and the platelet count was within normal range (241x10g/L). Procalcitonin of 1,43ng/mL and C-reactive protein of 86,2mg/L.

Electrocardiography revealed normal sinus rhythm, 86 bpm, normal axis and no acute ST-T wave changes.

Anaerobic and aerobic blood cultures were positive after 10 hours and culture on blood and chocolate agar plate with presence of 5% carbon dioxide at 37ºC. After two days of incubation a small Gram-negative bacillus was cultured and has been identified as A. actinomycetemcomitans.

Transoesophageal echocardiogram showed vegetation in the anterior leaflet and prolapse of the posterior leaflet of the mitral valve causing severe insufficiency. Thus, the diagnosis of subacute infective endocarditis was confirmed.

Chest and abdominal computed tomography with no evidence of septic emboli.

The patient was started on intravenous ceftriaxone and gentamicin. Given the severity of mitral insufficiency, the patient underwent valve replacement with a mechanical prosthesis, being discharged with normal functioning prosthesis and globally conserved systolic function.

Discussion: The diagnosis of invasive A. actinomycetemcomitans infection must be established as soon as possible in order to prevent possible complications. In this case, it was delayed due to the indolent clinical course, a non-specific presentation and a slow growth of this organism.

|P20

EXTERNAL QUALITY ASSESSMENT PILOT STUDY FOR THE DETECTION OF CANDIDA AURIS

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Aim: Aiming to raise the awareness about the risk of misidentification associated to this species, the Portuguese External Quality Assessment Program (PNAEQ), in collaboration with the national reference laboratory for parasitic and fungal infections of the National Institute of Health Dr. Ricardo Jorge, organized a pilot study in 2020 to evaluate the ability of Portuguese clinical microbiology laboratories to correctly identify C. auris.

Methods: Test samples contained suspensions of 3 yeast species (C. auris, C. duobushaemulonii, C. krusei). Samples were distributed to 18 participant laboratories for the identification of yeasts up to the level of the species, according to the method in use by the participant laboratory.

Results: The participation rate in the detection of C. auris scheme (94%).

Four different methods were used for species identification: automated biochemical method (10/17), mass spectrometry – MALDI-TOF (5/17), non-automated biochemical method (1/17) and culture – chromogenic media (1/17).

Regarding the instruments, three were used: Vitek 2 (10/17), Vitek MS (3/17) and Bruker biotyper (2/17). The remaining two laboratories used other non-instrumental methods.

The species C. auris was correctly identified by 88% (15/17) of the laboratories. Participants with incorrect/missing answers used manual methods.

Candida duobushaemulonii was correctly identified by 82% (14/17) of participating laboratories the species. Participants with incorrect answers used manual methods and automated methods Vitek 2.

All the participants (17/17) correctly identified the sample containing C. krusei.

Discussion/Conclusion: Since C. auris is considered an emergent pathogenic agent due to its multi-resistant phenotype, fast identification is mandatory for implementing measures to stop the dissemination.

The majority of the participating laboratories use automated biochemical methods or MALDI-TOF MS, with the updated database for C. auris.

Participants using non-automated methods such as API and culture in chromogenic media reported incorrect results for the identification of C. auris and C. duobushaemulonii.

The identification of yeasts to the species level is of the utmost importance in Hospital units, but also in the laboratories that handle ambulatory samples.

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MRSA BACTEREMIA: A 10-YEAR EXPERIENCE AT A TERTIARY CARE CENTER

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Introduction: Methicillin-resistant Staphylococcus aureus (MRSA) is a microorganism frequently associated with healthcare infections, contributing to increased morbidity and mortality. In intensive care / intermediate care units, we can find patients with increased risk factors for colonization / infection by MRSA, and these units should be considered a priority in the control of endemic MRSA.

Objective: Identify the relationship between MRSA bacteremia and prior MRSA colonization screening, over a 10-year period, in a tertiary hospital.

Methods: Retrospective review of MRSA blood culture isolates and screenings in Intensive Care and Intermediate Care units in a tertiary hospital, from 2010 to 2020.

Results: There were 466 patients with Staphylococcus aureus bacteremias; of those, 186 (39.9%) had bloodstream infections due to MRSA. Besides a decreased incidence of MRSA bacteremias over the study period, it was also observed a decline of the overall positivity of MRSA screening (2010: 15.9%; 2020: 1.4%). The average age was 72.0 years old (minimum: 3 months old; maximum: 92 years old), 63% male, and 53.8% (n = 100) admitted to an intensive care unit. Upon admission, 23% had positive MRSA screening. Of the screened patients, only 6.3% developed MRSA bacteremia. About 40% of patients with MRSA bacteremia had no admission screening test. All isolates were sensitive to vancomycin and in only 3.5% of the cases, resistance to mupirocin was detected.

Conclusion: This study corroborates the decreasing incidence of MRSA bacteremia cases and the need to maintain the practice of screening measures, epidemiological surveillance and infection control. The low resistance to mupirocin in isolated strains supports its use in nasal decontamination. MRSA screening can also identify colonized patients who might or

not benefit from empirical vancomycine therapy in the context of S. aureus bacteriemia, saving unnecessary use of broad spectrum antibiotics. Rates of bloodstream infection by MRSA are an indicator of quality of care and prevention and awareness among healthcare professionals is currently the most effective measure for controlling hospital infections and the emergence of new resistant strains.

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DID THE CARBAPENEM RESISTANCE INCREASE DURING THE COVID-19 PANDEMIC IN OUR INSTITUTION?

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Introduction: COVID-19 arose in Wuhan, China, on December 8, 2019. In Portugal, the first case was detected on March 2, 2020, and in our institution on March 13, 2020.

Many studies have reported a decreased incidence of several infectious respiratory diseases; however, many reports have described an increase in multidrug-resistant organisms (MDRO) during the COVID-19 pandemic.

Carbapenem resistant Enterobacterales (CRE) are an emerging MDRO with serious clinical and therapeutic implications. Its major resistance mechanism is the acquisition of carbapenemases, especially the KPC enzyme, which has been extensively reported in Klebsiella pneumoniae (Kp).

Objective: Evaluate the incidence of CRE, especially in Kp, in the pre-COVID-19 era (between 2017-2019) and during the COVID-19 pandemic (from 2020 to the 2021 1st trimester).

Methods: We performed in all new admissions, a screening with rectal swabs (RS) to search patients colonized by CRE. The RS was inoculated into a chromogenic media (chromID CARBA SMART agar, bioMérieux) and the suspected colonies were analyzed by an immunochromatographic test (NG-Test CARBA 5, biotech) for the identification of the major carbapenemases (KPC, NDM, IMP, VIM and OXA-48) and tested for carbapenem resistance using the Vitek 2 system (bioMérieux). All isolates from single patients recovered from infectious sites were also studied. We analyzed the trend of CRE colonization and infection during the time studied.

Results: We report an increasing rate of Carbapenem resistant Kp producers since 2017, with the highest incidence rate in 2021. The number of colonized and infected patients increased significantly during the last years. The most common CRE identified was KPC-Kp producers, followed by OXA-48-Kp.

Conclusions: Considering great infection control measures in the COVID-19 era, we would have expected a clear reduction in CRE acquisition, but this did not happen. In fact, the incidence of CRE acquisition went from 14% in 2017, to 23% in 2020 and 35% in 2021.

High rate of antibiotic utilization, variable rate of co-infection due to multiple morbidities, prolonged hospitalization, physical space limitations, constrained availability of personnel, shortages in personal protective equipment and many critically ill patients are some factors that contribute to this higher rate. Therefore, active surveillance of SARS-CoV-2 infected individuals for MDRO will be crucial.

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SYSTEMIC FUNGAL INFECTIONS: 8-YEAR RETROSPECTIVE ANALYSIS IN A NORTHERN PORTUGAL HOSPITAL

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The number of fungal infections has increased and it's believed this expansion has to do with an increase in the number of immunocompromised patients. This type of infections is also a reality when dealing with critically ill patients. Different species of fungi can be isolated and generally, systemic fungal infections have poor prognosis.

We selected all fungal isolates from positive haemocultures and cerebrospinal fluid cultures, from January 2013 to March 2021. A total of 81 patients were identified, most of them with fungemia by Candida sp (n=73) and 8 with Cryptococcal meningitis.

Contrary to what has been described in the literature, in our hospital, we could not verify an increase in the number of systemic fungal infections throw-out the years and we found 2016 to be the year with most isolates (18,5%). 81,5% of patients were committed, most in the internal medicine award (25,9%), followed by intensive care unit (22,2%) and oncology award (14,8%). The majority of patients (70%) were more than 60 years old and the prevalence in male patients was slightly superior (58%) to women (42%). The case fatality rate was about 54%.

All cases of Cryptococcal meningitis were human immunodeficiency virus (HIV)-infected patients, in 7 of them (Total n=8) it was an inaugural diagnosis. In 5 patients, Cryptococcus was also isolated in haemocultures.

Regarding the cases of fungemia by Candida sp, the most common isolation was C. albicans (52,1%), followed by C. glabrata (20,5%) and C. parapsilosis (20,5%), C. krusei, C. tropicalis and C. stellatoidea were also isolated in a small percentage of patients (2,7; 2,7 and 1,4 respectively). 80,8% of this candidemias were nosocomial infections, 79,5% of patients were being treated with antibiotics and in 74%, bacterial infection was present, mainly urinary tract infections (50%) and bacteraemias (27,8%), a large group also had an hospital stay of more than 20 days (76,7%). Among the risk factors identified were diabetes (27,4%), chemotherapy (21,9%), hepatic cirrhosis (8,2%) and 6,9% were undergoing immunosuppressive therapy.

This retrospective analysis reflects the importance of the awareness to this infection agents, especially in immunocompromised patients with a long hospital stay, even when bacterial agents are identified, as they are responsible for a high fatality rate.

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PREVALENCE OF RESPIRATORY VIRUS INFECTIONS DURING COVID-19 PANDEMIC

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Introduction: The SARS-CoV-2 pandemic started on December 8, 2019 in Wuhan, China. Since then, its spread throughout the world has been exponential. In Portugal, the first case was detected on March 2, 2020. In our hospital the first case was detected on March 13, 2020.

Objective: To better understand the etiologies of influenza-like syndromes in the COVID-19 period, we analyzed the samples taken in our hospital, between September 24, 2020 and March 3, 2021 with the request of respiratory panel.

Methods: Combined nasopharyngeal and oropharyngeal swabs specimens from suspected respiratory patients admitted to the emergency room and inpatients were collected in universal or viral transport media. We employed the BioFire-FilmArray Respiratory Panel 2.1, a multiplex, nucleic acid amplification platform that detects 22 viral and bacterial respiratory pathogens including SARS-CoV-2. We calculated the means and proportions to describe the distribution of positive patients per age group and sex and also the distribution of respiratory viruses.

Results: During the time studied, we analyzed 534 naso and oropharyngeal swabs from 455 patients, 136 women (30%) and 319 man (70%). In total, 43% (195) of the samples were positive, 71% (139) from males and 29% (56) from females. The average age among the positive patients was 62 years old. 46% of the positive patients were above 70 years old. The prevalence of SARS-CoV-2 was 36.7% (167 patients) and 34 patients (7.5%) were positive for other respiratory pathogens. SARS-CoV-2 co-detection was observed in 3.1% of cases (6/195). Of the non-Covid-19 viruses, the most frequently detected were Rhinovirus/Enterovirus (6.4%). The Influenza virus was not detected.

Conclusions: During the time when the SARS-CoV-2 epidemic hit Portugal with the 2nd and 3rd waves, seasonal respiratory viruses quickly disappeared while COVID-19 affected more than a third of patients with respiratory influenza-like illness in our hospital. These results show that the COVID-19 public health interventions (social distancing, use of masks and lock-downs) are having a beneficial impact on the prevention of other respiratory diseases.

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HUMAN HERPESVIRUS 6 MENINGOENCEPHALITIS – A CASE REPORT

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Introduction: Human herpesvirus 6 (HHV-6) belongs to the subfamily Betaherpesvirinae and consists of the species HHV-6A and HHV-6B. Like other herpesviruses, infection is very prevalent in humans and is associated with clinical latency. Initialinfection usually occurs between 6 and 24 months of age and may be asymptomatic or cause mild clinical manifestations, such as fever and/or exanthema subitum. We report a clinical case of an immunocompetent child with rare atypical clinicalmanifestations of a probable initial infection by HHV-6.

Clinical Case: A 2-year-old male child with a history of previous hospitalization for prematurity, posthemorrhagic ventricular dilation and placement of ventricoperitoneal shunt at birth, with no history of exanthema subitum, is brought to the emergency department by afebrile convulsive crisis, associated with generalized tonic-clonic movements, which initially responded well to diazepam, but quickly relapsed, requiring intubation and administration of phenytoin and levetiracetam. After clinical stabilization, a brain computed tomography was performed with no abnormal findings and, later, lumbar puncture, having been admitted to the Pediatric Intensive Care Unit. The cytochemical study of cerebrospinal fluid revealed 10 mononuclear/mm3, glucose = 0.85 g/L and proteins = 1.15 g/L, and the microbiological study revealed a nucleic acid amplification test (NAAT) by real time polymerase chain reaction positive for HHV-6. After 4 days of supportive treatment, the patient had a favorable outcome.

Conclusion: Since it is a neurotropic virus, the initial infection by HHV-6 can trigger rare atypical clinical manifestations involving the central nervous system, therefore this etiological agent cannot be neglected, specially if other causes have been ruled out. It is also important to note that NAAT result does not distinguish between initial infection and HHV-6 reactivation.

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PASTEURELLA SPP INFECTIONS: A CENTER EXPERIENCE

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Introduction: Oral flora of dogs and cats may contain several zoonotic pathogens and Pasteurella multocida has been reported as one of the major bacteria leading to human infection following animal bites [1]. The most common clinical presentation resulting from direct inoculation is cellulitis and lymphangitis [2]. Bacteremia is rare but carries a significant mortality rate [3, 4]. We related a clinical case, selected all pasteurella spp isolates from January 2013 to March 2021 and compared our results with literature.

Case Report: A 86-year-old man, with no relevant personal history, was admited in hospital for fever, pain and swelling in his right forearm, following a domestic cat scratching 7 days ago.

Laboratory tests only showed a considerable increase in Reactive C Protein, 34.5 mg/dL [<0.5 mg/dL].

The patient was diagnosed with cellulitis and was prescribed empirically a 10 days course of Amoxicillin/Clavulanic Acid and Clindamycin.

Positive blood cultures were subcultured onto Blood agar, MRSA agar and MacConkey's agar. On the first one, grew small opaque and grayish colonies, with no growth in the others agars. Gram-negative cocobacilli were observed in direct exam. The APINH test was inconclusive and Gram-negative chart on Vitek2 (Biomerieux®) detected Pasteurella multocida. The manual Antimicrobial Susceptibility test was performed according to EUCAST guidelines, with Amoxicillin/Clavulanic Acid, Levofloxacin and Sulfamethoxazole/Trimetropim discs and Penicillin strip to which the microorganism showed sensitivity. The patient was evaluated in an external consultation and local finding showed improvement.

Discussion /Conclusion: Since 2013, 20 cases of Pasteurella infection were reported in our hospital isolated from following samples: respiratory (n=17), exudate (n=2) and peritoneal (n=1). Only three of them was associated with bacteremia. The prevalence in male patients was slightly superior (60%) to women (40%).

Most of them (n=11) were treated, half with a combination of amoxicillin and clavulanic acid, the recommended treatment regimens[5]. This patient rapidly developed cellulitis at the site of injury, similar to what it is described in literature, and developed bacteremia, although is not usual.

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EIKENELLA CORRODENS: AN UNUSUAL LABORATORY FINDING WITH HIGH PATHOGENICITY - CLINICAL CASE

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Introduction: E. corrodens is a Gram negative, facultative anaerobic bacillus which belongs to the family Neisseriaceae. This fastidious organism is grouped together with another slow-growing organisms known as the HACEK group.

E. corrodens is a commensal of the human mouth and upper respiratory tract.

The recognition of this bacterium is important, as it is an unusual laboratory finding in routine cultural examinations in aerobiosis, with clinical importance due to its high pathogenicity.

Case description: Female patient, 62 years old, Caucasian, with personal history of bronchial asthma.

She went to the Emergency Department with pain in the right hypochondrium accompanied by fever and episodes of productive cough.

Two blood cultures were collected and a chest CT scan, which was compatible with right pleural effusion and pneumonia.

Thoracentesis was performed, which allowed a collection of pleural fluid sent for microbiological examination.

Afterwards, inoculation was carried out using enriched non-selective culture media (Columbia agar + 5% sheep blood - COS and Chocolate agar: CHOC - PVS), with bacterial growth occurring after 48 hours of incubation.

In the direct examination of Gram stain, Gram negative and rare polymorphonuclear bacilli were observed.

Biochemical tests demonstrated oxidase-positive and catalase-negative.

Subsequently, the identification was performed by the automated method on the Bruker MALDI Biotyper® equipment which revealed E. corrodens and the manual Antibiotic Sensitivity Test (AST) was realized.

The two blood cultures were positive with the same microorganism.

Posteriorly, the manual Antibiotic Sensitivity Test revealed sensitivity to ampicillin, cefotaxime, gentamicin and ciprofloxacin and resistance to clindamycin, which permitted clinicians to target antibiotics.

Discussion: E. corrodens is biochemically inactive for most of the tests and it is a microorganism that needs prolonged incubation time to grow making it difficult to identify.

In this clinical case, we intend, to emphasize the importance of a fast diagnosis to improve the prognosis of the patient that was made possible by the automated method on the Bruker MALDI Biotyper® equipment.

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LABORATORY PERFORMANCE EVALUATION OF MALARIA MORPHOLOGICAL IDENTIFICATION IN EQA PROGRAMS

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Introduction: Malaria, one of the main worldwide health diseases, is caused by Plasmodium species being of utmost importance the correct identification of each Plasmodium spp. Since 1995, the National Program for External Quality Assessment (PNAEQ) has implemented a Parasitic Morphology program which aims to evaluate the performance of participant laboratories in the identification of parasitic structures. To continually improve their performance, PNAEQ, in collaboration with a work group, provide updated scientific reports, courses and, when needed, implement corrective actions.

Objective: Evaluate the participant's performance on Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale identification, from 2011 to 2018.

Methodology: Among three annual rounds, the program provided blood smears (control samples) with instruction letters and result forms to each participant to perform the identification. Then, PNAEQ received and analyzed the results using the test from Excel Office 365 program. For each species and year, results were grouped by laboratory type (outpatient or hospital) and their performance were considered satisfactory ≥ 60% and unsatisfactory < 60%.

Results: Statistical analysis revealed a higher performance for P. falciparum identification, with outpatient laboratories showing satisfactory results in 2011, 2017 and 2018, and hospital laboratories in 2011 and between 2016 and 2018. The performance of P. malariae identification was satisfactory for outpatient laboratories in 2015 and 2017 and for hospital laboratories between 2015 and 2018. However, unsatisfactory results were observed in 2012, 2014, 2016 and 2018 for outpatient laboratories (2% - 56%) as in 2012 and 2014 for hospital laboratories (10% and 27%). Regarding P. ovale identification, results were mostly unsatisfactory, except in 2011 revealing satisfactory results in each laboratory types.

Conclusion: Malaria as a serious disease imply a correct identification of Plasmodium spp. in order to guide the clinicians into the adequate therapy. These results showed that the laboratory capacity for parasites identification differ across the three species. Thus, the participation in EQA programs is crucial for the continuous improvement of laboratory performance since not only monitor the quality of results but also assist in the adequate corrective measure to apply.

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A RARE CASE OF NOCARDIA SPP. ISOLATION IN CEREBROSPINAL FLUID IN A SEVERE COVID- 19 PATIENT

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Introduction: Nocardia spp. are Gram positive, variably acid-fast, catalase positive, filamentous branching, and strictly aerobic bacilli. Isolation of these organisms from clinical specimens doesn't always indicate disease. Rarely, in immunocompromised patients, hematogenous dissemination from a primary infection site can occur, with very poor prognosis and high mortality rates. We present a case of central nervous system (CNS) Nocardiosis in a Covid-19 infected patient admitted to an intensive care unit (ICU).

Clinical Case: A 63 years old smoking male, with arterial hypertension, presented to the hospital complaining of fever, dry cough, and headaches for 2 days. SARS-CoV-2 molecular testing was positive, and he was admitted to the ICU for sepsis and respiratory failure with associated cardiac distress, initiating corticotherapy and ventilatory support. 3 days later his neurological state deteriorated, and a cranioencephalic CT scan revealed pre-pontic and interpeduncular subarachnoid arterial bleeding. A lumbar punction for cerebrospinal fluid (CSF) was performed, and the specimen sent for microbiological evaluation. CSF Gram staining was apparently amicrobial. The specimen was inoculated in brain-heart infusion (BHI) broth, and blood and chocolate agar and incubated at 37ºC. After 48hours BHI was subcultured to blood agar; scarce, yellow, and wrinkled colonies appeared on blood agar and BHI-blood agar; Gram stain showed fine filaments, and acid-fast stain (modified Zhiel-Nielssen) was performed, showing acid-fast rods. By the time identification of the isolated colonies (through matrix-assisted laser desorption ionization time-of-flight mass spectrometry) was available, confirming Nocardia spp. infection, the patient had already deceased.

Discussion: The clinical presentation of CNS Nocardiosis is variable, with no specific signs and symptoms to guide the diagnosis; also, the laboratory work-up is challenging and time-consuming. A high clinical suspicion is mandatory and the microbiologist´s role is crucial for the correct diagnosis. The immunocompromised state of the patient, as well as cortico-therapy, are important factors in the disease. Before appropriate therapy was initialized, the neurological condition of the patient rapidly deteriorated and led to a poor outcome.

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SARS-COV-2 IN A MILITARY HOSPITAL

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Objective: The objectives of the present study are to analyze the SARS-CoV-2 polymerase chain reaction (PCR) results from all the patients from our hospital, between March 1, 2020 and March 31, 2021, studying the requests, positive samples, prevalence during all the months, cycle threshold (Ct) distribution and population characteristics.

Methods: A retrospective study was carried out. Combined nasopharyngeal and oropharyngeal swabs specimens from suspected respiratory patients admitted to the emergency room and inpatients were collected in universal or viral transport media. We employed two PCR equipments: a rRT-PCR from Cepheid (GeneXpert), the Xpert Xpress SARS-CoV-2, which is able to detect two genes: E and N (with Ct value) and the BioFire-FilmArray Respiratory Panel 2.1, a multiplex, nucleic acid amplification platform that detects 22 viral and bacterial respiratory pathogens including SARS-CoV-2 (with the detection of the S and M genes). We calculated the means and proportions to describe the distribution of SARS-CoV-2 per age group and sex. The positive results distribution during the time studied were evaluated and the Ct value of the GeneXpert results were analyzed according to the patient origin (inpatient or outpatient).

Results: During the time studied, we analyzed 4137 naso and oropharyngeal swabs from 2780 patients, 771 women (28%) and 2009 man (72%). In total, 19% (521) of the samples were positive, 71% (370) from outpatients and 29% (151) from inpatients. The average age among the positive patients was 58 years old. 38% of the positive patients were above 70 years old and mostly were men (70%). When we analyze the number of positive patients along the time of SARS-CoV-2 detection, we observe three waves, the 1st one in March/April, the 2nd in October/November and a 3rd one in January/February. According to the Ct distribution, the outpatient's values (22.5) were lower than the inpatients (30.1), which mean that they have a higher viral load.

Conclusions: Most of our COVID-19 patients were males, under 70 years old and came from the emergency room. The distribution of positive patients along the time study is similar of Portugal distribution (3 waves and the same period). The viral load of SARS-CoV-2 outpatients are higher, which would reflect a more aggressive symptomatology.

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INCIDENCE AND EPIDEMIOLOGY OF NONTUBERCULOS MYCOBACTERIA IN THE CATCHMENT AREA OF CENTRO HOSPITALAR TONDELA-VISEU, 2017-2019

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Introduction: Nontuberculous mycobacteria (NTM) have shown a rising incidence worldwide causing substantive disease burden, with pulmonary infection as the main clinical manifestation. Many patients develop persistent chronic infection, and drug resistance is frequent, as treatment requires prolonged multidrug therapy.

Objectives: To report the incidence rate of NTM cases in the catchment area of Centro Hospitalar Tondela-Viseu, the frequency and diversity of NTM species, and their distribution according to the source sample, gender and age of patients, between 2017 and 2019.

Material and Methods: A retrospective analysis was performed involving patients with a positive cultural exam for acid-fast bacilli, either obtained directly from BACTECTM Mycobacteria Growth Indicator Tubes (MGITTM 960) or from Löwenstein-Jensen slanted tubes (BIO-RADTM). Negative samples for Ag MPT64 were sent for identification at the National Reference Laboratory.

Results: The overall NTM incidence rate was 6.3/100.000 inhabitants (7.8, 4.1 and 7.1 in 2017, 2018 and 2019 respectively). Nine different species were identified from 51 isolates, with slow growth NTM representing 86%. The most frequent isolated species were M. avium complex (MAC) (39.2%), M. *lentiflavum* (25.5%), M. *gordonae* (9.8%) and M. *kansasii* (5.9%). Other species included M. *chelonae*, M. *simiae*, M. *fortuitum* and M. *scrofulaceum*. All isolates were obtained from respiratory samples (57.7% sputum and 32.7% broncho-alveolar lavage). Affected patients had a median age of 70 years, 59.6% were male, and structural lung disease was frequent at presentation (69%). A pure first culture was obtained in 24 isolates, 15 in a second culture and 12 from Löwenstein-Jensen cultures. Only 1/51 cases were detected by an acid-fast smear from the primary sample.

Conclusions: During the study period the incidence rate of NTM remained stable (average of 6.3) and in line with data from reviewed literature, being mainly driven by slow growing species of MAC, which are dominant in Portugal. It is also important to highlight the unique high frequency of M. *lentiflavum*, which is mostly non-pathogenic, and the absence of M. *abscessus* cases. The difficulty in detecting NTM in primary acid-fast smears showcases the low mycobacterial load present, which could be linked to asymptomatic infection or chronic and slow progression of lung disease.

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YERSINIA SPP.: A 20-YEAR RETROSPECTIVE STUDY FROM A TERTIARY CENTER

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Background: Yersinia spp. is a Gram-negative, facultative anaerobic, coccobacillus member of the Enterobacteriaceae family. Clinical presentation varies from self-limited abdominal symptoms to fulminant systemic infection.

Materials/methods: Retrospective review of Yersinia spp. isolates at a Portuguese university hospital in the last 20 years. Several selective agar plating media were used for bacterial recovery, including MacConkey, Salmonella-Shigella and Yersinia CIN agar.

Results: A total of 37 Yersinia spp. isolates were characterized; 5 were excluded for missing data. Patients´ age ranged from 6 mo. to 88 yo.; most were children (59.4%), male (56.3%) and presented during winter months. Isolates were recovered from fecal specimens (71.8%), blood (18.8%), urine (6.3%) and peritoneal fluid (3.1%). Y. enterocolitica (96,9%) was the most common isolate, followed by Y. pseudotuberculosis (3.1%). Clinical features at presentation included diarrhea (59.4%), abdominal pain (34.4%), fever (31.3%), neurological symptoms (12.5%), arthritis (3.1%) and urinary symptoms (3.1%); extraintestinal manifestations were only found in adults. Pediatric isolates had lower antimicrobial resistance rates. Most cases were treated as outpatients and responded favorably to medical therapy. One patient with secondary hemochromatosis, unrecognized at admission, underwent surgical intervention for acute appendicitis suspicion. Two deaths occurred in immunocompromised patients.

Conclusion: Yersinia spp. infection is an increasingly recognized cause of morbidity and mortality, but clinical diagnosis is challenging. In pseudo-appendicitis syndrome, distinctive surgical findings can be suggestive, but confirmation by culture is required. However, isolation may be difficult with standard media and require specific media/incubation features. Our center performs Yersinia spp. screening not only upon clinical suspicion, but also routinely in all pediatric fecal samples since 2018, successfully increasing its recovery.

Clinicians should consider this cause of gastroenteritis and pseudo-appendicitis, specially in patients with iron overload, and request appropriate microbiologic testing. In most cases antibiotic therapy will lead to rapid clinical improvement and obviate the need for surgery.

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URINARY TRACT INFECTION: PREVALENCE AND SUSCEPTIBILITY PROFILE TO ANTIMICROBIALS IN A COMMUNITY LABORATORY

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Objectives: Evaluate the etiological spectrum and the pattern of resistance to antimicrobials of the main agents responsible for urinary tract infections (UTI) in 2020.

Methodology: 3872 urines were analyzed. Positive urines (N = 1138) were evaluated for the etiologic agent and the susceptibility profile to antibiotics using the Vitek 2 Compact system (bioMérieux). Amoxicillin, amoxicillin/clavulanic acid, cefuroxime, cefotaxime, ertapenem, ciprofloxacin, *fosfomycin*, gentamicin, cotrimoxazole and nitrofurantoin were the antibiotics studied.

Results: The prevalence of UTI was 29.4% (17% in males and 83% in females). The most frequent agents were Escherichia coli (57.9%), Klebsiella pneumoniae (15.4%), Proteus mirabilis (6.5%) and Enterococcus faecalis (3.7%). For *Enterobacterales*, the antibiotics with higher resistance were amoxicillin, amoxicillin/clavulanic acid, ciprofloxacin and cotrimoxazole. Fosfomycin showed a resistance rate of 35% for K. pneumoniae. For E. coli, the frequency of resistant strains was lower than for K. pneumoniae. E. coli presents high susceptibility to *fosfomycin* and nitrofurantoin, 99% and 100%, respectively. E. faecalis showed no resistance to amoxicillin and nitrofurantoin. Resistance to 3rd generation cephalosporins by the production of expanded-spectrum beta-lactamases (ESBL) was 26.3% and 8.2% for K. pneumoniae and E. coli, respectively. Regarding resistance to carbapenems, three multiresistant strains (K. pneumoniae producing *carbapenemases*-KPC) were detected.

Conclusions: E. coli remains the most frequent UTI agent in the community. Amoxicillin, fluoroquinolones and cotrimoxazole are highly resistant, demonstrating their low effectiveness as empirical therapy. In E. coli, the antibiotics that showed the least resistance were *fosfomycin* and nitrofurantoin, which may be a good option for the empirical treatment of uncomplicated UTI when caused by E. coli. The high resistance found for the other two bacterial strains reveals that caution should be exercised in the empirical prescription of antibiotics since E. coli is responsible for only 58% of UTIs. Multidrug resistance due to the production of ESBL or *carbapenemases* is more frequent in K. *pneumoniae*, being an emerging public health problem both at the hospital and in the community.

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OBSERVATIONAL STUDY: THE KINETICS OF ACQUIRED HUMORAL IMMUNITY IN HOSPITAL POPULATION VACCINATED WITH THE VACCINE ´´COMIRNATY´´ FOR SARSCOV2

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Objective: Understanding antibody kinetics after SARS-CoV-2 vaccination is important to evaluate vaccine efficacy and immune response durability.

In this study, we evaluated the kinetics for serologic response of vaccinated healthcare professionals (HP) to verify the compliance with the manufacturers claims and also the clearance of the RNA messenger (RS1) for spike S1 viral protein (S1).

Material and Methods: We assessed the serologic response in 42 vaccinated HP (8 male, 34 female); from these, 3 were excluded: 2 due to SARS-CoV-2 infection after 1st dose, and 1 did not conclude the study. A control group (CG) of 13 unvaccinated co-workers were tested to assure no group contamination at workplace with asymptomatic individuals occurred during the study.

4 samples were collected from the vaccinated group (VG): 15 and 21 days after Comirnaty dose one (CD1), and 21 and 60 days after Comirnaty dose 2 (CD2).

2 samples were collected from CG, corresponding to the 1st and 4th sample withdraws of the VG.

All subjects were tested for ACCESS SARS-CoV-2 IgM and ACCESS SARS-CoV-2 IgG (Beckman Coulter) against S1.

Results: All CG results remained negative during the study.

15 days after CD1 61% of VG developed some immune response; this value increased to 80% 21 days after CD1. At this time 41% still had IgM.

21 days after CD2, 100% of VG had immune response and 77% still had IgM.

60 days after CD2, 100% of the subjects had IgG response and only 15.4% had IgM.

Unrelatedly of age and sex, we observed a group of higher responders (HR) with 10-33 U/L IgG (n=13) and a group of lower responders (LR) between 1.2-9.9U/L (n=18), 21 days after CD1.

21 days after CD2 we observed a group of HR 30-57 U/L IgG that almost tripled (n=30) and the LR with 10-29 U/L IgG (n=10) almost reduced to half.

60 days after CD2, we observed 50% IgG titles reduction for the HR group and 75% IgG reduction for the LR group.

Conclusion: Negative serologic results of CG indicate low probability of workplace massive contamination of the VG with SARS-CoV-2.

The observed serology kinetics 60 days after CD2, shows at least 84,6% of VG with no stimuli for antibody production and thus completely cleared for RS1. .

A massive loss of 50%-75% of IgG antibodies between days 21 and 60 after CD2, suggests that humoral response may not last and that cellular response can probably give a better memory immune defense against SARS-CoV-2.

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VALUE OF KAPPA FREE LIGTH CHAIN AS A BIOMARKER IN CSF ANALYSIS FOR MULTIPLE SCLEROSIS DIAGNOSIS IN THREE CENTERS FROM PORTUGAL

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Introduction: The increase of the kappa free light chain in CSF of MS patients has been reported in several publications that had evaluate the value of K-index as a surrogate marker for the gold standard method for the determination of intrathecal synthesis of immunoglobulins, the detection of oligoclonal bands (OCB) in cerebrospinal fluid (CSF)(Leurs et al., 2020).

Goals: Evaluate the prognostic value of kappa free light chain and kappa index as biomarker for the results of the OCB tests, and asses its performance in the diferencial diagnosis of multiple sclerosis.

Methods: 199 paired CSF/serum samples from three different centers in Portugal, to which the OCB testing was requested were included. K-FLC was determined by turbidimetry (Freelite in Optilite, Binding Site). Statistical analysis was performed with the GraphPad Prism8 software.

Results: MS patients had a higher K-FLCCSF concentration and K-index (median:74,24) than the non-MS group (K-FLCCSF median: 3,77mg/L vs 0,3 mg/L) (K-index median: 74,24 vs 0,52). K-FLCCSF concentration in the samples with OCB positive was higher than in the samples with OCB negative results, 4,4 mg/L and 0,3 mg/L respectively, as well as the K-index, 70,24 and 0,52. A K-FLCCSF concentration <0,31 mg/L obtain in 86 samples (43,2%) showed a NPV of 97,7% for negative OCB. ROC analysis of K-index values vs BOC retrieve an area under the curve of 94,6% and versus MS diagnosis of 94,9 %. Such results are well above the ones obtain for the IgG-Index vs BOC and MS diagnosis, 76,1% and 76,9% respectively.

The previous published K-index cut-off of 6,6 had a sensibility of 94,4% and a specificity of 83,4% for MS diagnosis, in line with the reported by the authors (sens. 93% and spec 83%)(Leurs et al., 2020).

Conclusions: Our findings confirm the prognostic value of K-FLC as a biomarker for BOC results and MS diagnosis and can be integrated in an algorithm for MS screening that can help to reduce the volume of OCB determinations.

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IMMUNOGLOBULIN D MULTIPLE MYELOMA: AN UNCOMMON DIAGNOSIS

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Introduction: Immunoglobulin D Multiple Myeloma (IgD MM) is rare and represents circa 2% of all myeloma subtypes. It usually attains younger patients, has an aggressive course with multiorgan involvement and is resistant to chemotherapy. Renal failure is very common and survival outcome is poor.

Case Report: We present a case of an IgDMM in a 58-year-old man; with unremarkable past medical history, who went to the emergency department with intense lumbar bone pain, respiratory distress, and fever.

Laboratory investigation showed anemia with hemoglobin of 8,5 g/dl; erythrocyte sedimentation rate (ESR) 122mm, and elevated creatinine (2,97mg/dL), urea (141mg/dL), alkaline phosphatase (263 mg/dl), calcium (2,67 mmol/L) and LDH (460 mg/dl). Urinary analysis revealed proteinuria and few hyaline-granular casts. Serum free light chain (FLC) lambda level was 1300mg/dL, free light chain kappa 1,16mg/dL, κ/λ ratio <0,26; IgA 13,8mg/dL; IgG 501mg/dL; IgM 6,2mg/dL; kappa chain 100mg/dL; lambda chain 390 mg/dL. Serum electrophoresis and immunofixation revealed presence of Lambda Light Chains. The presence of isolated and the excessive Lambda Light Chains with no correspondent Heavy Chain, forced further study. IgD level was 506 mg/dL. Anti-IgD monoclonal antibody was used for immunofixation analysis and an IgD/Lambda monoclonal band was identified. Urinary immunofixation detects one paraprotein correspondent to FLC Lambda. The bone marrow biopsy was positive for plasma cells (15%). Lumbar spinal imaging showed a cortical osteolytic lesion of L1 and small dorsal lesions. Renal biopsy demonstrated cast nephropathy, requiring hemodialysis.

Started chemotherapy treatment, however suspended secondary to complications. The patient died 2 years after diagnosis.

Conclusions: This patient presented anemia, bone lesions with hypercalcemia, renal insufficiency and analytical alterations compatible with the diagnosis of MM. This case demonstrates that IgD MM can be associated to an excessive production of FLC and can be wrongly diagnosed as MM of FLC. Awareness of this rare subtype of MM and its epidemiological, clinical and immunochemical characteristics is important to establish the exact diagnosis.

|P37

ELEVATED β-HCG DIFFERENTIAL DIAGNOSIS: THE ROLE OF THE CLINICAL PATHOLOGY LABORATORY

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Introduction: Human chorionic gonadotropin (hCG) is an important biomarker for detecting and monitoring various physiologic and pathologic conditions, including pregnancy, pregnancy-related disorders and malignancies (I.e., gestational trophoblastic disease and germ cell tumors). Immunoassays are commonly used to determine the β subunit of hCG (β-hCG); however, these are susceptible to analytical interference causing false-positive (FP) results. Laboratory identification of FP or true results can assist in guiding clinical management.

Case Report: 36-year-old woman, with history of Non-Hodgkin Lymphoma in remission, presents with progressive elevation of serum β-hCG levels for two months (β-HCG 370 > 2728 mUI/mL; Access Total βhCG 5th IS, Beckman Coulter). Intrauterine pregnancy was excluded by transvaginal ultrasound. Exploratory laparoscopy was performed with excision of a formation suggestive of extrauterine gestation on the right ovary; the anatomopathological study did not reveal the presence of trophoblast cells or chorionic villi. Imaging exams did not corroborate the diagnostic hypothesis of a germ cell tumor. The biochemical study, including α-fetoprotein, progesterone, LH, FSH, estradiol, prolactin, ACTH and IGF1, was also not suggestive of neoplastic hypothesis. The Clinical Pathology laboratory was contacted to assess the existence of potential interferences in the hormonal assay. Pre-analytical interference, namely drugs, were excluded. Urinary β-hCG measurement was suggested, which was positive (318mUI/mL), ruling out FP results due to heterophile antibodies. An immunochromatographic hCG test (Dedicio) and a free-β-hCG assay (38,12 IU/mL; BRAHMS Free βhCG Kryptor, ThermoScientific), using a different methodology, were performed; both were positive. This allowed the exclusion of analytical interferents.

Subsequently, it was decided to perform a PET-TC with 18FDG, which revealed the presence of 3 pulmonary hypermetabolic foci. The patient is waiting for an Oncology consultation.

Conclusion: In patients in whom common causes of elevated β-HCG have been excluded, the Clinical Pathologist plays a critical role in guiding clinical management, either by identifying FP results or confirming true results, as well as by suggesting alternative tests to achieve a diagnosis.

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SIX SIGMA METHODOLOGY TO EVALUATE AND IMPROVE THE RESULTS OF THREE PARAMETERS OF CLINICAL CHEMISTRY EVALUATION QUALITY ASSESSMENT PROGRAM

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Introduction and Objectives: From the PNAEQ's Clinical Chemistry Program, three analytes were chosen to evaluate the quality assessment: Total Cholesterol, LDL Cholesterol and Triglycerides. Results from 2018 to 2020 were studied to evaluate and develop actions to improve the participants' Sigma quality level.

Methods: The method selected to evaluate and improve the program's results is DMAIC (define, measure, analyse, improve, control). To calculate the Sigma quality level, two approaches were used. The first only considers inaccuracy, obtaining the Sigma quality level through the calculation of defects per million opportunities (DPMO). The second approach relies on a model that assesses both inaccuracy and imprecision based on data from EQA programs and was only used for Total

Cholesterol and Triglycerides analytes and for 2020's results. Both compare the laboratory results to the consensus values of each sample and both determine the Sigma quality level considering specifications based on biological variation.

Results and Discussion: Through these approaches it was possible to determine the mean Sigma quality levels by parameter and year, based on the desirable level of biological variation. For the Total Cholesterol analyte, the results from the first approach were 2,23, 2,07 and 2,01, from 2018 to 2020, respectively. The results for the LDL Cholesterol were 1,46, 1,64 and 2,63 and for the Triglycerides parameter 3,14, 3,31 and 3,31, respectively. With the second approach, the results from the year 2020 were 2,38 and 7,67 for Total Cholesterol and Triglycerides analytes, respectively. When comparing Sigma levels, it is essential to specify the method and level of biological variation.

After the Define and Measure phases, the Analyse step identifies potential causes for the low performance and organizes them by priority. In the Improve step, improvement actions are developed and implemented. The Control intends to maintain the results of the improvement actions.

Conclusions: The Six Sigma methodology presents a structured approach to problem solving not only in a manufacturing environment but also in the service sector. When implemented within external quality assessment programs, it provides a metric that allows laboratories to compare their performance with each other and implement and maintain the suggested improvement actions.

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ALTERATIONS IN COVID-19 PATIENT'S URINE SEDIMENT

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Introduction: Renal tubular cells are rarely present in urine sediment of healthy individuals. Their presence has been described in acute renal tubular diseases due to ischemia or toxicity. Ischemic nephropathy may be seen following trauma, shock or sepsis. These pathological conditions are also accompanied by an increase casts and red blood cells in urine studies.

Aims: To compare the urine sediment examination in a population of patients with and without SARS-COV2 infection at-hospital setting.

Population and Methods: Urine sediment examination was performed in patients admitted to a hospital.

The study of urine sediment was performed by flow cytometry using the Sysmex UF-4000 equipment, allowing different cellular elements to be categorized based on size, complexity and affinity for specific fluochromes. For the statistical study we used the SPSS program and the U-Mann Whitney test considering significant the value of p<0.05 si="">95%).

Results: We studied 512 patients admitted to a hospital between September 2020 and February 2021.

Patients were divided in two groups – with and without active SARS-CoV2 infection.

Group A- Covid-19 patients (n=389): 154 female, median age 80 years old (47-96) and 235 male, median age 74 years old (28-94).

Group B - Non-Covid-19 patients (n=123): 43 female median age 82 years old (71-95) and 80 male, median age 73 years old (22-89). The following table describes the flow cytometry findings and the statiscal study.

		Red Blood Cells µl	White Blood Cells µl	Pyocyte µl	Epithelial cells µl	Renal tubular cells µl	Casts µl
Group A	Median	18,55	18,8	0,0	10,95	5,2	0,8
	Mínimum	0,4	0,2	0,0	0,4	0,0	0,0
	Máximum	42354,5	18280,8	1213,9	549,0	82,2	74,61
Group B	Median	12,5	25	0,05	7,5	3,5	0,675
	Mínimum	0,1	0,5	0,0	0,0	0,0	0,0
	Máximum	25815,6	17687,3	1238,8	107	98,1	58,96
p	0,003	0,11	0,842	<0,001	<0,001	0,007	

Discussion of Results: The urine sediment of COVID-19 patients (A) had a higher number of epithelial (p<0,001) and renal tubular cells (p<0,001) than the non-COVID-19 patients group (B), which was statistically significant. The presence of red blood cells (p=0,003) and casts (p=0,007) was also higher in COVID-19 patients group, which was also statiscally significant. These results suggest renal tubular lesion in the context of SARS-CoV2 infection.

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URINALYSIS PROTEIN/CREATININE RATIO AS A SCREENING TOOL

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Introduction: Proteinuria is an important indicator of a wide variety of kidney conditions, some pathological (p.e., pre-eclampsia, multiple myeloma) and other benign (p.e., pregnancy, intense activity, orthostatic disorder). Urine dipstick is a widely available test which gives us useful clinical information.The protein/creatinine ratio (PCR) is measured on a spot urine sample and is a useful alternative to 24-hour proteinuria measurements. A negative PCR has been shown to effectively rule out pathologic proteinuria in suspected cases while a positive result should lead to further investigation.

Objective: In this retrospective study we aim to compare the PCR from a quantitative method (Roche cobas® 8000) with a semi-quantitative urinalysis (Siemens Clinitek Novus® PRO 12) and evaluate the latter's performance.

Materials and Methods: For this study the pair of samples were selected between March 1 and April 10, 2021. Proteinuria and creatininuria were determined on Roche cobas® 8000 through immunoturbidimetry and colorimetry based on the rate-blanked Jaffé method, respectively. Urinary protein and creatinine were assessed on Siemens Clinitek Novus® PRO 12 through reflectance spectroscopy. For each sample, the PCR was calculated and compared with the urine dipstick results using the >500 mg protein/g creatinine cutoff for kidney damage found in literature. For the statistical analysis, Microsoft Excel® and the R® programming language were used.

Results: In a total of 418 samples, 231 belonged to female and 187 to male patients, with an average age of 51,82 years. Of these, 179 were found to be above the previously defined cutoff value and were considered as pathological. The area under the receiver operating characteristic curve value was 0,88. When evaluating at a 500 mg/g value, the urine dipstick sensibility and specificity were 82,6% and 86,7%, respectively, with a positive and negative predictive value of 82.1% and 87,0%, accordingly.

Conclusion: Urinalysis is commonly performed for detecting proteinuria, which implies a wide range of diagnostic possibilities. Given our performance evaluation, the urine dipstick PCR may have a role for primary screening of kidney disease.

Declaration of Conflict of Interest: All authors declare, on their honor, no conflict of interest.

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TOTAL LIGHT CHAIN QUANTIFICATION IN LIGHT CHAIN MULTIPLE MYELOMA – CRITICAL APPRAISAL

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Introduction: Light Chain Multiple Myeloma (LCMM) represents around 15% of all multiple myeloma (MM) cases and is characterized by the overproduction of light chains (LC) by malignant plasma cells.

International Myeloma Working Group (IMWG) recommends serum free light chains (sFLC) quantification in the screening, monitoring, and prognosis evaluation of LCMM. However, total light chain quantification (TLCq) is still used in laboratory practice in this context.

Immunoglobulins (Ig) are glycoproteins produced by plasma cells that have two LC and two heavy chains (HC).

TLCq methods quantify both bound and sFLC, while sFLC methods use antibodies that recognize epitopes present in the constant region of the LC, which are hidden when joined to an HC but are exposed when in their free form.

In order to achieve the best possible outcomes, early and accurate detection of the disease is a crucial factor. Our aim is to understand the current role of TLCq as a disease marker in LCMM, in comparison with sFLC quantification.

Materials and Methods: We compared TLCq and sFLC concentrations and ratios at diagnosis, in a cohort of 36 patients diagnosed with LCMM between 2016 and 2021.

sFLC were measured using the turbidimetric assay Freelite® by BindingSite. The reference values (Ref) are 0.33-1.94 mg/dL for Kappa (K) sFLC, 0.57-2.63 mg/dL for Lambda (L) sFLC and 0.26-1.65 for free K/L Ratio (KLR).

TLCq was performed by the nephelometric assay IMMAGE® by Beckman-Coulter. The Ref are 629-1350 mg/dL for K TLCq, 313-723 mg/dL for L TLCq and 1.53-3.29 for total KLR.

Results: Our population was divided according to the monoclonal LC at diagnosis.

sFLC KLR accurately identified the monoclonal LC on 36 (100%) patients.

On the other hand, TLCq only identified clonality on 6 (32%) K LCMM patients, and 15 (88%) L LCMM patients. 68% of K LCMM had the total KLR within the Ref, which suggests the absence of clonality.

Conclusion: This study has demonstrated that TLCq has a poor performance on clonality detection in the diagnosis of LCMM. For this reason, whenever possible, sFLC should be the assay of choice in these situations, as recommended by the IMWG guidelines.

According to the presented results, the necessity of TLCq in the current laboratory workout should be assessed.

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STATISTICAL ANALYSIS OF THE ANNUAL SOROLOGICAL RESULTS OF COXIELLA BURNETII BY THE INDIRECT IMMUNOFLUORESCENCE METHOD

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Introduction: Q fever (Zoonosis) is a systemic disease caused by Coxiella Burnetii, an obligate intracellular gram-negative bacterium, which can lead to atypical pneumonia, febrile syndrome, hepatitis or endocarditis. In other words, in its acute and self-limited form, it usually has a benign prognosis, while in its chronic, usually localized form, the evolution of the disease is serious and potentially fatal.

The diagnosis is based on serological methods due to the difficulty in isolating the agent.

Purpose: Q fever is considered to be an underdiagnosed zoonosis due to its non-specific symptoms. This work aims to understand which are the medical services that most require serological analysis, which are the signs / symptoms / hypotheses of diagnosis that are the basis of this research, as well as to know the amount of positive results found in the different aspects analyzed by serology.

Materials and methods: Retrospective analysis of all serologies requested for Coxiella Burnetii from 1/1/2020 to 12/31/2020. They were performed using the indirect immunofluorescence assay (IFA) kit for the determination of IgG and IgM antibodies phases I and II in serum or plasma samples from patients.

Results: The total number of requested and realized tests was 250, being 38.4% (96) of pediatric age and 58% (145) of the total in males. The majority of requests were from internal medicine services of the hospital center, 27.2% (68), highlighting the number of requests made by the infectious diseases services 8% (20) and intensive care units 5.2 % (13). In total we had 25 patients (10%) with some positive result, with IgG phase I, 12 patients, IgM phase I, 10 patients, IgG phase II, 16 patients and IgM phase II, 14 patients.

Conclusion: IFA interpretation provided valuable results for serological diagnosis of acute and chronic Q fever. That means that the follow-up using this method is essential, although it is difficult to identify the optimal timing, frequency and duration

due to various antibody reactivity profiles, being always necessary to consider interferences and technical problems. In patients with chronic Q fever, prompt diagnosis and early treatment might be lifesaving.

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IMMUNE RESPONSE 21 DAYS AFTER THE FIRST ADMINISTRATION OF THE BNT162B2 (BIONTECH/PFIZER COMIRNATY) VACCINE IN HOSPITAL HEALTHCARE WORKERS

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The COVID-19 pandemic has put all the scientific community to work with a common goal, which is to understand the physiopathology of the disease in order to be able to control it worldwide. The development of vaccines was a vital step in this direction, however, evaluating the humoral immune response after vaccination is crucial to determine whether the vaccine is effective and how long lasting are its effects. The objective of this study is to evaluate the serological levels of the IgG antibodies against the SARS-CoV-2 spike protein, 21 days after the first administration of the BNT162b2 vaccine in hospital healthcare workers (HHW). Blood samples were collected from 642 HHW. Serums were subjected to the Abbott's quantitative anti-spike IgG chemiluminescent assay on the Alinity equipment. The results were compiled and analyzed in an Excel table. By quantifying the SARS-CoV-2 anti-spike IgG, we verified that a small percentage (9%) had significantly higher values than the others. By complementing the testing with the analysis of SARS-CoV-2 anti-nucleocapsid IgG in the samples from subjects without previous diagnose and anti-spike values above 5000 UA/mL (46), we realized that about half (48%) had already a previous contact with the virus, since they had a positive anti-nucleocapsid IgG. This suggests that their immune response was not solely due to the vaccine. When analyzing the cases in which the diagnosis was already known (14), we found that about half (43%) did not have an anti-nucleocapsid IgG, possibly due to the fact that the levels of anti-nucleocapsid IgG decrease over time eventually reaching undetectable values. Therefore, this serological study allowed us to realize that the fact that an individual having previous contact with the virus is decisive in the immune response after the first dose of the vaccine. Additionally, we found that an IgG anti-spike value above 5000 UA/mL indicates about a 48% probability that there has already been previous contact with the virus. However, in the remaining 52%, it cannot be infered that this is not true, since in previously diagnosed cases, we found that about 43% no longer had detectable values of IgG anti-nucleocapsid, due to antibodies kinetics. Further studies are suggested in order to understand if having had COVID-19 will influence the long-term immunity levels.

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IMMUNOGLOBULIN D MEASUREMENT BY TWO DIFFERENT IMMUNOASSAYS

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In serum, immunoglobulin D (IgD) normally occurs in trace amounts, with a reference value ≤10 mg/dL. Measuring IgD is most useful in monoclonal gammopathies, particularly in those 1% that secrete this particular immunoglobulin. In these patients, serum IgD levels can serve as a biomarker to monitor the disease .

Our objective was to assess the concordance between IgD measurement in an Optilite ® system (The Binding Site - TBS), using a latex-enhanced immunoturbidimetry kit from TBS, and in an Immage ® 800 Protein Chemistry Analyzer (Beckman Coulter), by a non-competitive kinetic Near Infrared Particle Immunoassay (NIPIA), using a kit from Trimero Diagnostics.

For that purpose, we selected 46 serum samples from patients with a suspected or confirmed diagnosis of multiple myeloma. Those were obtained from June 2020 to February 2021.

IgD was measured in both systems. Levels in a range of 0.1 – 2220 mg/dL were obtained with Image ®. For Optilite ®, the range was 1.3 – 2048 mg/dL. These results reflect values spanning from close to the limit of detection to antigen excess. Half of the samples had measurements above the reference value.

Data analysis was performed on GraphPad© software. A Passing-Bablok regression showed a slope of 0.92 (0.8988 – 0.9439 with a 95% confidence interval). Moreover, amongst the 46 samples, there were 3 patients with multiple measurements (that is, in the context of follow-up), whose evolution through time was identical using both systems. A R^2 of 0.99 was obtained, which means that these 2 methods showed a proportional variation.

Although more robust studies would be needed to accurately evaluate an equivalence between methods, our results suggest a very good correlation between the results obtained in both systems. Furthermore, Optilite ® shows operational advantages when antigen excess is detected, as it automatically calculates and performs dilutions as needed.

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SEROLOGICAL STUDY OF 2 HEALTH PROFESSIONALS INFECTED WITH SARSCOV 2 AFTER FIRST DOSE OF COMIRNATY VACCINE

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Objective: Specific anti-SARS-CoV-2 antibody testing play a vital role in the post infection and vaccine elicit immunity determination. Correlation between serological response of infected (IS) and vaccinated subject (VS) was evaluated in this study.

Case report: We compared a serological response of two subjects, both infected by SARS-CoV-2 after 1st dose Comirnaty´s vaccine and 39 not infected VS. The results were obtained 60 days after infection or after the 2nd dose Comirnaty´s vaccine, for IS and VS respectively.

IS were diagnosed with Covid-19 using GenExpert Xpert®Xpress Sars-Cov-2 PCR (Genes E and N2) test.

All subjects were tested by using ACCESS SARS-CoV-2 IgM and ACCESS SARS-CoV-2 IgG (Beckman Coulter) against spike S1 viral protein (S1).

IS 1: 36-year-old female presented with mild sore throat (2 days), diarrhea (11 days), rhinorrhea (5 days) and ageusia (3 days). Without other relevant symptoms or medical needs. Serological results: IgG - 1.55 S/CO and IgM - 0.24 S/CO.

IS 2: 49-year-old male with clinical symptoms of strong cough, high axillary temperature but no fever (1 day of 37,8ºC) medicated with paracetamol, severe headache (3 days), eye and nostrils pain, anosmia and ageusia (3 days), abundant rhinorrhea (3 days), mild to severe diarrhea (5 days) with hemorrhoids. Serological results: IgG - 19.40 S/CO and IgM - 1.44 S/CO.

The VS was divided in 3 groups according their IgG response (in S/CO):

1. 18,1% had 1-10; 2. 45,5% had 10 - 19.9; 3. 36,4% had 20 - 36.75.

Discussion: In the reported cases, we found examples of two individuals infected with Covid-19 with different levels of clinical and serologic antibody responses, 60 days after infection.

The mild symptomatic IS, lacked IgM response and kept low IgG antibodies against SARS-CoV-2 S1, approximately corresponding to the less responsive group of VS.

The more severe symptomatic IS also developed a stronger serologic response with higher levels of IgG approximately corresponding to the VS group with higher response.

Our case study suggests that there is a higher player on immune response to avoid the development of more severe clinical sintoms for Covid 19 disease, probably the immune cellular response, and thus should be evaluated on the total immune response for both IS and VS.

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TRACKING ASYMPTOMATIC SARS-COV-2 INFECTION WITH SEROLOGY TESTING IN A HOSPITAL HEALTHCARE WORKERS POPULATION

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Hospital healthcare workers (HHW) are at increased risk of developing COVID-19 due to occupational exposure to infected patients and if infected, can transmit the virus to patients and co-workers. Since HHW with asymptomatic infection are not indicated for PCR testing, they are never diagnosed. Individuals who have had COVID-19 develop specific antibodies against SARS-CoV-2 and these persist after active infection, so serological tests can be a useful method to detect asymptomatic cases. To maximize professional's surveillance and minimize infection´s transmission we defined a protocol with the Occupational Health Unit, that consisted in a periodic serologic evaluation of SARS-CoV-2 antibodies. This evaluation was conducted among HHW at designated COVID-19 healthcare facilities. Therefore, the objective of this study is assessing if the tracking of COVID-19 through serology testing is efficient in a hospital healthcare population. Blood was collected monthly from 306 HHW. The anti-nucleocapsid IgG and anti-spike IgM serum semi-quantitative SARS-CoV-2 tests were performed with Abbott´s chemiluminescence tests on the Architect equipment. The results were compiled in Excel and the acceptance criterion for a case to be considered detected by serology was to have no previous positive PCR for SARS-CoV-2 and to have at least a positive nucleocapsid IgG. 90% (275/306) were both IgG and IgM negative and 10% (31/306) were positive for at least one of the immunoglobulins. Of the 10% of positive cases, 52% (16/31) had both positive immunoglobulins, 26% (8/31) had only positive anti-spike IgM, and 22% (7/31) had only positive anti-nucleocapsid IgG. After assessing whether these cases had previous positive PCR or not and if they had at least positive anti-nucleocapsid IgG, 4% (14/31) were cases detected by serologic tests, 3% (8/31) were previously known cases of COVID-19 and 3% (9/31) were unclear cases. It is possible to infer that approximately half (14/31) of the seropositive cases were detected due to this study, which correspond to asymptomatic carriers. These results are congruent with the published data. In conclusion, in periods of high community dissemination and high prevalence, periodic serological monitoring of COVID-19 increases the effectiveness of the containment strategy.

|P47

COMPARISON BETWEEN THE DIFFERENT MODELS USED TO SET ANALYTICAL PERFORMANCE SPECIFICATIONS

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Introdution: The analytical specifications in laboratory medicine are used to assess the performance of their analytical methods, being an essential part of internal quality control. The Consensus Agreement made in the Stockholm conference, defines a hierarchy of the different models used to establish the analytical specifications.

The purpose of this work is to compare the most frequent used models to establish set analytical performance specifications in laboratory medicine.

Materials and Methods: An internet search was carried out on reference sites using the keywords: ´´analytical specifications´´, ´´quality control´´ and ´´clinical laboratory´´ in Portuguese and English.

Results:

MODEL	ADVANTAGES	DISADVANTAGES
Biological Variation	There are specifications set for a great number of parameters;	Does not take into account the state of health of the patient and associated pathologies;
	Greatly supported by the scientific community.	Need of standardization in the acquirement methodology of the values.
Clinician's opinion	It takes in consideration the opinion of the professional who will take actions based on the results.	There are a great number of variables associated with each parameter.
		Highly dependent on the group of medical doctors chosen.
Legislation	The values obtained could be used as minimal specifications.	Reduced number of parameters for which there are specifications.
State-of-the-art	The specification values are extracted of situations in which the laboratory is evaluated (External Quality Control Programs).	The sample material is artificial, so it does not fully reflect the precision and deviation compared with a patience sample.

Conclusion: The most widely used model for obtaining analytical specifications worldwide is biological variability, followed by the state of the art.There is still no gold standard with regard to the choice of specifications, being the model's choice made according to the specific situation of each laboratory. It should be noted that the quality specifications are not watertight and immutable parameters, and should be changed whenever necessary, for a continuous improvement of quality.

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IMMUNOLOGIC RESPONSE OF HEALTHCARE PROFESSIONALS VACCINATED WITH THE PFIZER-BIONTECH COVID-19 VACCINE: A FOLLOW-UP STUDY

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Objectives: To evaluate the immunologic response of Pfizer-BioNTech vaccine (tozinameran) in healthcare professionals (HCPs) six months after the primary vaccination series (1 or 2 doses) and after a booster vaccine dose. The protection against SARS-CoV-2 infection within this period was also assessed.

Materials & Methods: Serum levels of IgG antibodies against the receptor-binding domain of the SARS-CoV-2 spike protein S1 subunit were determined using the SARS-CoV-2 IgG II Quant kit (Abbott) at different time points. In this follow-up study, three instants were assessed: T2, 180 days after the first vaccine dose; T3, at the time of the booster dose; and T3+1, 30 days after the booster dose. The cutoff value was 50.0 AU/mL. Positive results were stratified in 3 titer probability curves of neutralizing antibodies: 51-2999, probability <90%; 3000-6299, 90-95% probability; ≥6300, probability ≥99%.

Results: A total of 1861 HCPs were included in the study: average age, 41.6±10.9 years; 79.2% female; 779 took all three doses. At T2, antibody titers were available for 1592 HCPs: 99.6% positive. HCPs with 2 vaccine doses and Covid infection showed higher antibody levels than those who did not had disease (p<.001) or those who received 1 vaccine dose (with previous infection or not; p<.001). At T3, serologic levels were distributed as: 0.1% ≤50; 92.9% 51-2999; 4.7% 3000-6299; 2.2% ≥6300. HCPs who received 2 doses and had Covid infection presented elevated antibody titers (25% ≥6300), which were significantly higher than those with 2 vaccine doses without infection (1% ≥6300; p>.001). At T3+1, 100% HCPs tested positive (96.1% ≥6300), independently of previous infection. Notably, a longer time between the second and the booster dose (155-211 vs. 274-351 days) produced higher antibody levels (p=.002).

Conclusions: HCPs maintained a strong immunologic response six months after initiating the primary vaccine series with the Pfizer-BioNTech vaccine. Moreover, HCPs who completed the primary vaccination scheme and had been exposed to Sars-Cov-2 infection presented higher titers of neutralizing antibodies. Our data also shows that a longer period between the primary vaccine series and the booster dose (>274 days) results in a higher antibody response, which may provide important insights for optimizing COVID-19 vaccination guidelines.

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VARIATIONS IN HAIRY CELL LEUKEMIA-VARIANT: TWO CASE REPORTS

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Hairy cell leukemia-variant (HCL-V) is a rare type of chronic leukaemia, accounting for approximately 0.4% of chronic lymphoid malignancies. We report two cases of HCL-V with different stages of clinical presentations and different immunophenotypic profiles.

Case 1: A 75-years-old man, ex-smoker, with multimorbidity including type 2 diabetes and chronic kidney disease, performed a routine blood count test, revealing lymphocytosis. On the peripheral blood smear, atypical lymphocytes were observed, featuring hairy cytoplasmic projections and a prominent nucleoli. The immunophenotypic analysis revealed a population of lymphocytes expressing CD11c and FLAIR-1. They were negative for CD103 and CD5. Surprisingly, these cells didn't express any light chain. The diagnosis of HCL-V was considered. Currently, the patient is asymptomatic, and he is not receiving any treatment for HCL-V. However, he undergoes periodic laboratory tests to monitor the disease.

Case 2: A 90-years-old man with a 6-year history of HCL-V presented with ascites and bilateral lower extremity edema. He was not receiving any treatment for HCL-V but he was undergoing periodic tests to monitor the disease. Splenomegaly was previously reported but splenectomy was not considered due to his advanced age.

A paracentesis was performed, revealing a high proportion of mononuclear leukocytes. Hairy cells with a prominent nucleoli were identified in the cytologic analysis. The same cells were observed in the peripheral blood smear. The peritoneal effusion immunophenotypic analysis revealed a population of lymphocytes expressing CD19, CD103, CD11c, LAIR1 and kappa light chains. Heart failure, portal vein thrombosis and cirrhosis were excluded.

Discussion: These cases reveal two different stages of HCL-V. The first one, is an early stage that was incidentally diagnosed during a routine blood test, highlighting the importance of clinical and laboratory suspicion of this disease. The second case shows ascites as a rare late-stage complication of HCL-V. The mechanism behind the peritoneal effusion remains unknown.

In both cases, immunophenotypic analysis was a key tool for the diagnosis. The first case is particularly interesting because it shows an unusual immunophenotypic profile in which the hairy cells didn't express neither lambda nor kappa light chains.

In conclusion, this work shows two unusual presentations of HCL-V that should be taken into account when considering this diagnosis.

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PRIMARY EFFUSION LYMPHOMA IN AN AIDS PATIENT: A CASE REPORT

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Primary effusion lymphoma (PEL) is a rare large B cell lymphoma, usually presenting as serous effusions (pleural, peritoneal and/or pericardiac), without associated tumour mass. It has a strong association with herpesvirus 8 (HHV8), and frequently co-infection with Epstein–Barr virus (EBV1). Due to its rarity, we thought it would be relevant to share this case.

We report a case of a 47-year-old, man, with a medical history of mixed HIV ½ infection and with AIDS criteria (CDC C3), diagnosed in 2010, and with Kaposi's sarcoma in 2011, presenting to the emergency department (ED) with weight loss, anorexia, and progressive increase of the abdominal distension and constipation, over the last 3 weeks. The ultrasound

confirmed the presence of ascites. He was admitted to the hospital for further study. During the ED stay, it is noteworthy to report the analysis of ascitic fluid, with 21600/μL leukocytes and “observation of 60% of large cells, lymphoid-like, prominent deeply basophilic cytoplasm, and several vacuoles, suggestive of high-grade non-Hodgkin’s lymphoma”. During hospital stay, it is noteworthy the ascitic fluid’s immunophenotype: CD45+, CD19-, CD20-. It was initially excluded some types of B-cell Non-Hodgkin lymphoma, like Burkitt lymphoma and diffuse large B-cell lymphoma. Further study was done, and it was performed diagnostic laparoscopy. The ascitic fluid was sent to Clinical Pathology and Anatomical Pathology (AP). The AP report was: "The cells are large, with irregular hyperchromatic nucleus, some with nucleoli. The cells are CD45+; CD20-; CD79A; CD3-; AE1;AE3-; CD5-; HHV8 strongly +" These characteristics are compatible with Primary Effusion Lymphoma. The patient was referred to a hematologist-oncologist and is under therapeutic protocol.

Primary effusion lymphoma is a rare, aggressive disease with a poor prognosis. The role of the clinical pathologist is fundamental because they actively participate in the diagnosis of this disease. Even though the diagnosis was not made within the field of Clinical Pathology, the pathologist had a contributing role in the differential diagnosis.

|P51

URIANALISYS – THE OPTIMIZATION LABORATORY WORK FLOW AND CLINICAL PRESCRIPTION OF ANTIBIOTICS

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Urinary Tract Infections (UTI) are one of the more recurrent pathologies in hospitalized and outdoor patients. The ITU represents a clinical problem and has an important impact in the increase of bacterial resistances[1,2] and a high sanitary cost due to the take of antibiotics. In most of the cases, this infection is characterized by the presence in urine of high counts of bacteria and leukocyturia. The culture exam remains the gold standard laboratory test for the etiological diagnosis of ITU. However, the definitive results are only available 48 hours after the collection of the sample[3].

The aim of this study is to evaluate a rapid method of screening in order to separate the negative from the positive urinary samples.

We performed a retrospective study between October and December 2021. The data collected included, dipsticks, fluorescent flow cytometry (FFC), particle digital imaging (Automated Urine Analysis in Sysmex Modular Solution UN -3000 ™ - UC-3500; UF-5000; UD-10) and bacteriological exam results.

A total of urine samples from 7964 patients (hospitalized and outdoor) were the object of this study. In 5710 samples (71,7%) microorganisms (bacteria and yeast like cells) were not identified by FFC and the

microbiology results were reported like negative (78,6%) or with no clinical relevance (21,4%) because the culture presented 3 or more different microorganisms. 2254 samples were reported by FFC with the presence of microorganisms. Among these, 956 with the existence of Gram Negative Bacilli (GNB) by cytometry and 897 had a microbiological exam with >10^5 CFU (Colony Forming Units) of BGN.

This study will provide, in a near future, the implementation of new procedures aiming to discard negative samples and report both, negative and positive ones, to the Physician. This rapid information (few minutes), using an automated screening method, represents an important benefit for the patient, with no need of antibiotic treatment (in negative samples) or the empirical prescription mainly concerning the infection agent (in positive samples).

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|P52

EVALUATION OF PERFORMANCE OF PNAEQ PARTICIPANTS IN MYCOBACTERIOLOGY SCHEME 2016-2020 AND COMPARISON WITH PREVIOUS RESULTS (2007-2015)

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Objective: Evaluate laboratories performance in Mycobacteriology - microscopy program provided by National External Quality Assessment Program (PNAEQ) – comparison between 2 periods (2007/2015 – 2016/2020) and study of COVID-19 impact.

Methodology: During 2007-2015 it was sent 89 positive and 72 negative slides of mycobacteriology microscopy and in 2016-2020 41 positive and 34 negative. The results were compared with the expected results and re-evaluated by the organizing Laboratory in case of discrepancy. This study included national public and private clinical laboratories as well as laboratories from Portuguese speaking countries (CPLP).

The COVID-19 impact was made using the chi-squared test considering a significance level of 5%.

Results: In the first period analysed (2007-2015) there were 3,76% incorrect results corresponding to: quantification error (0,55%); false negative (2,27%) and false positive (0,94%) in 4817 results (89 positive [(8)1-9/100 fields; (38) 1+;(32) 2+;(11) 3+] and 72 negative).

During 2016-2020 there were 6,36% of incorrect results: quantification error (2,49%); false negative (2,49%) and false positive (1,38) in 1525 results (41 positive [(20) 1+; (16) 2+; (5) 3+] and 34 negative).

The samples analysed in pre-pandemic period (2017-2019), 24 positive [(11) 1+;(11) 2+;(2) 3+] and 21 negative, presented 94,54% correct results and 5,46% incorrect results – quantification error (1,98%); false negative (2,36%) and false positive (1,13%).

The samples analysed in the pandemic period (March 2020 - April 2021), 17 positive [(9) 1+;(5) 2+;(3) 3+] and 13 negative, presented 91,63% of correct results and 8,37% incorrect results – quantification error (3,65%); false negative (2,79%) and false positive (1,93%).

Significant differences between pre-pandemic and pandemic period was observed (p=0.03)

Conclusion: We observed a decrease in performance through the studied period, accentuated during the pandemic period, mainly with the increase of quantification error and false positive.

Comparing the performance from 2007-2015 with 2016-2020 we observe a decrease of performance during the recent years, also with an increase in quantification errors.

The decrease of performance observed in the last 4 years (2016-2020) reinforces the importance of participating in external quality assessment schemes and practical education.

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STREPTOCOCCUS PNEUMONIAE SEPSIS IN CHILDREN AFTER INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINES

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Key-words: Sepsis, Streptococcus pneumoniae, Children

Introduction: Streptococcus pneumoniae causes invasive (IPD) and non-invasive (NIPD) pneumococcal disease, mainly in the pediatric population. It is considered an uncommon causative agent of neonatal sepsis, including serious infections such as bacteremia, pneumonia, and meningitis; however, in this age group and before the age of 6 months, such infections can become fatal and have mortality rates that range from 1% to 14%. Since 2020, pneumococcal conjugate vaccine (PCV)-13 has been used in a 2-, 4-, and 12-month schedule as part of the universal immunization program - National Vaccination Program (NVP) 2020.

Aim: Case report of two children that could be a vaccine failure against S. pneumoniae.

Material and methods: S. pneumoniae was identified (Maldi-Tof, Bruker) on two children aged 5 months with bacteremia in blood stream culture. Whole genomic sequenciation was performed. Multiple Loci Sequence Typing (MLST), which is the most frequently used genotyping technique for S. pneumoniae. Antimicrobial susceptibility to penicillin, erythromycin and levofloxacin were determined by Vitek 2 (bioMerieux, France). MIC breakpoint interpretations were based on updated EUCAST-2022 standards.

Discussion and Conclusions: It was identified serotype 19F in one child. In this case S. pneumoniae is levofloxacin susceptible, increased exposure, penicillin and erythromycin resistant.

Another identified serotype was serotype 8. Serotype 8 isolate belonging to the 8-ST53 clone determinated by MLST. In this case S. pneumoniae is levofloxacine, peniciline and erytromicime susceptible.

The serotype is a key determinant of IPD potential and prevalence, however, analysis by MLST typing, contribute to the genetic characterization and understanding of their spread.

Currently, in countries of Western Europe in which the PCV13 vaccine is used, serotype 8 is one of the most frequently recovered, because it's not part of serotype polysaccharides (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) of the vaccine. These children fulfilled the Portuguese NVP 2020.

The incidence of pneumococcal sepsis in children remained high after the introduction of the pneumococcal conjugate vaccine (PCV)-13.

In conclusion, the first child appears to be a vaccine failure and the second does not, however, in both cases it caused invasive disease.

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THE CONTRIBUTION IN PROGNOSIS OF IL-6 IN PATIENTS WITH COVID-19

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Background: Since the late 1990s, serum levels of Interleukin-6 (IL-6) have been used as a diagnostic for several inflammatory diseases [1]. IL-6 is a pleiotropic proinflammatory cytokine produced by a variety of cells including lymphocytes, monocytes and fibroblastos [2]. Its serum values rise rapidly in response to various pathological events, and are usually associated with prognoses of a severe course [(3-5)]. Recently, the COVID-19 pandemic has elucidated another very important role played by this cytokine in the follow-up of patients, revealing that the dosage of IL-6 is able to guide the clinician regarding the severity of the disease and the eventual need for transfer to the Intensive Care Unit (ICU) [6]. SARS-CoV-2 infection induces a dose-dependent production of IL-6 from bronchial epithelial cells, causing systemic inflammation, leading to hypoxemic respiratory failure, which may be associated with the increased release of this particular cytokine. This biological phenomenon is called "cytokine storm"[7].

Methods: One-year retrospective study of serum IL-6 levels in 172 patients admitted to the ICU with SARS-CoV-2 at the our Hospital Center. The analytical method used was electrochemiluminescence immunoassay (ECLIA) using the cobas e 801 immunoassay analyzer.

Results: The higher levels of IL-6 in patients with more severe clinical conditions, admitted to the ICU, demonstrate the prognosis of these patients was worst and with a higher mortality rate than patients with lower IL-6 levels.

Discussion/ Conclusion: There is hypothesized that modulation of IL-6 levels or the effects of IL-6 may reduce disease duration and/or severity, proposed as the most accurate predictor of disease course as well as mortality in patients infected with SARS-CoV-2. Furthermore, therapeutic implications targeting IL-6 or its signaling, have shown success in treating patients with COVID-19.The assessment of IL-6 levels allowed controlling the severity of the disease, enabling adjustments to be made in the therapeutic strategies used.The greatest knowledge about IL-6 can contribute to the development of new therapeutic strategies and/or new biotechnological applications, mainly for the pharmaceutical industry, allowing for a deeper understanding of the physiological mechanisms of IL-6 in the face to the infection caused by SARS-CoV-2.

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PROCALCITONIN VARIATION AS A BIOMARKER OF BACTEREMIA IN SEVERE COVID-19

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The variation in Procalcitonin (PCT) has been widely studied as biomarker of infection, sepsis and to guide antibiotic management. During the COVID-19 Pandemic this biomarker was especially used by Intensive Care Units (ICU) to monitor patients positive for COVID-19 as many of these were prone to over-infection. Our goal is to evaluate the variation of procalcitonin in ICU patients with COVID-19 who developed bacteremia.

PCT was measured in 25 ICU patients who met the criteria in the period between 01-01-2021 and the 31-03-2021. Of these, 5 had only one measurement of PCT, and thus were excluded. For the 20 patients who met all criteria, 95% were male with a mean age of 65 years old. The mean value of PCT was 0,66 ng/mL, with overall mean of twelve determinations per patient. The highest value of PCT, in 50% of patients, was measured at the same time of bacteremia, in which, the most common isolate were Gram negative Bacili (60%).

We found that, the maximum value of PCT was timely associated with the diagnosis of bacteremia in 50% of patients with severe COVID-19. Initial reports have shown that most patients with COVID-19 didn't have elevated procalcitonin (>0.5 ng/mL) but elevated levels were found in severe cases and in patients with worse outcome, and bacterial co-infections.

Another finding was that Gram negative bacteria was most commonly isolated at the time of maximum PCT value. On the other hand, Coagulase negative Cocci were the most commonly bacteria isolated in the 10 patients in whom no association was found. In fact, as referred in literature, additional studies are needed to verify the putative bacterial origin of procalcitonin increase in patients with severe COVID-19.

Although, PCT is useful to stratify patients for severity of COVID-19 disease, there is no consentual opinion on this matter. For better evaluation of the variation of PCT and its value as a biomarker of co-infection a greater number of patients should be included and other parameters considered such as the antibiotherapy scheme, acute response factors and outcome.

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RELATIONSHIP BETWEEN MEASLES IMMUNIZATION COVERAGE, INTERFERON-Γ PRODUCTION AGAINST SARS-COV-2 AND M. TUBERCULOSIS IN HEALTH CARE WORKERS

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Introduction: The lack of immunity to SARS-CoV-2 has led to rapid evolution since its discovery in December 2019. Various researchers hypothesized that live-attenuated vaccines as rubella, measles, and Bacillus Calmette-Guérin (BCG) can result in cross protection against severe COVID-19. Measles vaccination is done by live-attenuated, negative-stranded RNA virus.

Measles vaccine is a lifetime vaccine. Pfizer/BioNTech vaccine (BNT162b2) is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine that encodes a perfusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein.

Aim: We described the correlation between measles IgG titres, Interferon-γ TB and interferon-γ production against SARS-CoV-2.

Material and methods: Health care workers (HCW) data of TB Interferon-γ, and measles IgG were used from a previous screening. Pfizer/BioNTech vaccinated HCWs were tested for IgG, and SARS-CoV-2 Interferon-γ. The Spearman rank correlation coefficient (rho) was used to determine the relationship between different variables. Data were analysed using SPSS software version 26.0. The statistical significance was considered when the P-value was < 0.05.

Results: A total of 532 were included, being 433 (81.4%) females. Interferon-γ TB was reported in 415 (78%) of HCWs, from that 390 (93.7%) presented measles IgG titres, and 344 (82.9%) also produced specific Interferon-γ SARS-CoV-2. All present at least one of the protections. A positive correlation was observed between IgG titres of SARS-CoV-2 and T-cell memory response against specific SARS-CoV-2 S-domain (rho=0.307; p<0.001). In other hand, a negative correlation was observed between interferon-γ against TB and cellular immunity to SARS-CoV-2 (rho=-0.112; p=0.014). No correlation was observed with measles immunity.

Conclusion: Our analysis provides novel insight into the potential correlation between the coverage rates of SARS-CoV-2 IgG titres coverage and cellular immunity against COVID-19 infection. High levels of IgG titres SARS-CoV-2 also linked to high values of IGRA SARS-COV-2. Measles immunization had no relation with both SARS-CoV-2 immunity analyzed.

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MIXED LYMPHOID/MYELOID LEUKEMIA IN PEDIATRICS - CLINICAL CASE

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Introduction: Leukemias are a rare type of neoplasms. They comprise a heterogeneous group of entities, with different clinicopathological characteristics. They may appear in pediatric age and present an aggressive clinical course.

With the development of diagnostic techniques, from the determination of the blood count, the morphological study of peripheral blood, immunophenotyping, cytogenetics and molecular biology, as well as the different therapeutic options, the rates of early detection and survival have increased.

Clinical Case: Male child, 12 years old, with no relevant personal history, seeks medical attention at the emergency department with marked asthenia, fever, dyspnea, nausea and vomiting. Upon clinical observation, he was conscious and oriented, with mucocutaneous pallor and poor peripheral perfusion. Analytically it was observed anemia (hemoglobin 3,2 g/dl), leukocytosis (47.17x103/mL), lymphocytosis (53% of lymphocytes; 25.00x103/mL) and elevation of the enzyme Lactate Dehydrogenase (2349 UI/L). In the determination of the blood count, using the Sysmex XN-1000 equipment, it was possible to visualize changes in the leukocyte scatter plot and alerts were issued by the equipment for the possible presence of blasts/atypical cells. A peripheral blood smear was performed, which showed marked lymphocytosis and the presence of about 34% of blasts.

In the immunophenotyping study it was revealed the presence of 72% of blasts with meyloid and B lymphoid immunophenotype, compatible with Acute Mixed B/Myeloid Leukemia (immunophenotype: CD3-, CD7-, CD13-, CD14-, CD19+, CD20-, CD24+, CD33-, CD34-, CD45+, CD58+, CD64-, CD81+, CD117-, CD123+, MPO+. DNA Index: 1.34; Ploidy: Hyperdiploid). A cytogenetic study of peripheral blood was performed, identifying the presence of a structural change on chromosome 14 (14q32, location of the IGH locus); a bone marrow sample was also studied, verifying the presence of the alteration in chromosome 14, and the presence of a deletion in the CDKN2A gene (9p21).

Discussion: Laboratory diagnosis in pediatric onco-hematology can be quite challenging. The aim of this case is to demonstrate the important contribution that the hemogram and the morphological evaluation of the peripheral blood smear can have in the initial diagnostic approach of these patients.

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AUTOIMMUNE HEMOLYTIC ANEMIAS: THE ROLE OF IMMUNOHEMATOLOGY LAB IN DIAGNOSIS AND TRANSFUSIONAL SUPPORT

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The study of hemolytic anemia represents a challenging endeavor to the immunohematology labs. Specific methodologies should be available to circumvent this fact.

We describe 3 clinical cases of Warm Autoimmune Hemolytic Anemia (WAIHA), Cold Autoimmune hemolytic Anemia (CAIHA) and Drug induced hemolytic anemia (DIIHA) in the clinical perspective and serological approach.

Case 1 (WAIHA): Male, diagnosis of Chronic Lymphocytic Leukemia (CLL) since 2013 – stage A Binet. In 2019 during an outpatient follow-up, he presented with severe anemia. Referred to Blood Banks for RBC transfusion. DAT and IAT positive and X-Match positive with RBC units with same phenotype as patient. Refer to clinician to use pre-medication prior to RBC transfusion.

Case 2 (CAIHA): Male, diagnosis of Hodgkin Lymphoma in 2019 that underwent a Alo transplant on 5/11/21 with donor O Rh – (conditioning TBI + FLU/ATG) . On Day+84, in an outpatient follow-up, he presented with worsening anemia (Hb 8,4 g/dL) and undetectable haptoglobin. DAT and IAT were positive. The ABO/Rh typing have pan agglutination, and X-Match is incompatible. Plasma and RBC were treated with Thiol agents. The serological interference was circumvented at 37ºC.

Case 3 (DIIHA): Female, diagnosis of germinal cell tumor of the hypophysis/optical quiasma, in 2002. CR after surgery + high dose chemotherapy and RT. Relapse in 2013 and 2º relapse in 2019 after AutoSCT. She was admitted in the ICU for sepsis from CVC and empirical antibiotherapy was started with Piperacilin/Tazobactam and vancomycin. Her condition worsens, with severe anemia (Hb 4,9 g/dL) and hemolysis. 2 units of pRBC were requested to the blood bank. Positive DAT and IAT with eluate negative. X-match positive. Coke - colored like plasma. Serological studies evolving drugs conducted to the suspension of Piperacillin/Tazobactam intake.

Exclude the presence of alloantibodies, not delay transfusion of RBCs and organize a SOP for the laboratory study of AIHA.

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PLASMA CELL LEUKEMIA - A CHALLENGING CASE OF PERIPHERAL BLOOD SMEAR EVALUATION

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Introduction: Plasma Cell Leukemia (PCL) is a rare and aggressive variant form of myeloma defined by the presence of peripheral blood plasmacytosis, accounting for >20% of the differential or >2x109/L of circulating plasma cells. In Europe, the incidence is 0.4 per million individuals per year. The prognosis is poor, with median survival below one year.

Case Report: We report a case of a 79-year-old male with a past medical history of IgG/lambda Monoclonal Gammopathy of Undetermined Significance (MGUS), with a stable course since 2002. In March 2022, he was admitted with a one-month history of confusion and agitation, with associated weight loss. Emergency room evaluation identified kidney failure, hypercalcemia and evidence of osteolytic lesions. Peripheral blood smear revealed 22% of plasma cells, with heterogeneous morphologic features: mature plasma cells with eccentric nucleus, abundant basophilic cytoplasm and a perinuclear hof, and atypical larger immature forms, with central nucleus, high nuclear-cytoplasmic ratio and, occasionally, evident nucleoli. Serum electrophoresis identified a monoclonal band in the gamma-zone (3.9 g/l), and flow cytometry analysis was consistent with an IgG/lambda PCL.

Conclusion: This case highlights how cytomorphology is crucial in the diagnosis of PCL. Collaboration between clinical pathologists and hematology clinicians is essential, given its aggressive course.

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FLAGGING PERFORMANCE EVALUATION OF THE BECKMAN-COULTER DXH900 HEMATOLOGY ANALYZER

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Introduction: Blast cell count is an essential part of the diagnosis and monitoring of acute leukemias. This count can only be reliably performed in manual blood smear microscopic examination. Modern hematology analyzers provide an alarm that a patient's blood may contain blast cells. This flag's performance is a crucial tool in reducing unnecessary manual blood smear examination, increasing the efficiency and accuracy in complete blood count validation.

Objective: In this retrospective study we aim to study the performance of the "Blast" flags given by the Beckman-Coulter DxH900 hematology analyzer.

Methods: A total of 17032 samples of a complete blood count performed by the Beckman-Coulter DxH900 were selected. The "Blast" flags reported by the equipment were compared with the manual blast cell count of the corresponding blood smears. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for the manual blast cell count cut-offs 1, 5 and 20 blast cells. Statistical analysis was conducted P14 BLAST with Microsoft Excel®.

Results: The sensitivity, specificity, PPV and NPV of the "Blast" flag for each cut-off were, respectively: 1 blast cell, 38.32%, 97.91%, 10.41%, 99.6%; 5 blast cells, 38.1%, 97.82%, 6.09%, 99.77%; 20 blast cells, 46.34%, 97.79%, 4.82%, 99.87%.

Discussion: Since the true prevalence of blast cells is unknown in the general population, our calculated NPV cannot be applied. However, in a population such as this study's (which includes hematology inpatients) this condition is expected to be more prevalent, leading the "real" NPV to be higher than reported.

Conclusion: Overall, the very high NPV for all defined blast cell count cut-offs shows that the absence of the "Blast" flag seems to be a suitable tool to confirm the dearth of blast cells in the patient's blood smear.

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TRANSFUSIONAL SAFETY AND PRE-ANALYTICAL SAFETY SYSTEMS - A PILOT ASSAY IN AN ONCOLOGICAL HOSPITAL

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The implementation of a transfusional security system was mandatory for Blood Banks and Transfusional Medicine Services. In our hospital we have adopted BTrac solution. These system includes the collection of a main sample for crossmatch

at bedside, identifying the patient using the wristband adopted in the hospital, and the application of transfusion identifying who performs the application, the vital signs and times of infusion. In our hospital we have adopted these solution and also the use of a routine sample for Complete Blood Count, because our patients performed them in daily basis. Thus we have two pathways to obtain samples that can be used to perform crossmatch if the patient needs transfusional support.

In order to use these sample of CBC an interface between BTrac and Clinidata was done and all the samples for analytical routine can be collected at bedside.

For achieving this endeavor, we organize, in collaboration of Pathology Laboratory team, all the pre-analytical phase in wards: collection of different samples at the beside of the patients, using the wristband, identifying the nurse and the time of collection of these samples. And we decided to implement LabTrac.

With this application we verify that an adaptation should be done. Instead of local of prescription equal to local of sampling we choose that local of sampling should be the local were the patient is at the moment independently of the local of prescription. This allow us to organize the sampling centered on patient and the different places were sampling are needed.

This solution is applied to in ward patients and outward patients with the exception of the Central place of sampling.

The main values we won were the standardization of all the collections, the organization of time of collection by nurses, very important for in ward patients, the use of patient wristband identification as the starting point for these pathway, and last but not the least safety for patients and personal.

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BURKITT´S LYMPHOMA - THE ROLE OF THE CLINICAL PATHOLOGIST

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Introduction: Burkitt´s lymphoma (BL) is a rare (1-2% of all lymphomas) non-Hodgkin lymphoma (NHL) with high proliferative rate, derived from germinal center B cells. Characterized by translocation and deregulation of the MYC gene on chromosome 8. The clinical presentation and frequency of Epstein-Barr virus infection varies according to the epidemiological subtype of BL (endemic, sporadic and immunodeficiency associated).

Sporadic BL is seen mainly in children and young adults, but there is also an incidence peak in elderly patients; more prevalent in the male.

BL is a highly aggressive but potentially curable tumour, with long-term overall survival in 70-90% of cases. However, there are several adverse prognostic factors.

We report a case of sporadic form of BL.

Clinical case: A 84-year-old man with medical history of heart failure, primary cutaneous follicular NHL, diagnosed in 2013 with complete response, and prostate adenocarcinoma.

Patient reports a need for help in activities of daily living in recent months; associated with anorexia, insomnia, headache and lumbar back pain.

Laboratory findings revealed anemia, leukocytosis of 96730/uL (neutrophils 67230/uL, lymphocytes 4840/uL, monocytes 2420/uL) and thrombocytopenia of 58,000/uL; elevation of LDH, uric acid, ferritin and CRP.

The peripheral blood smear showed a shift to the left of the granulocytic series with 1.5% of blasts and the presence of erythroblasts.

The myelogram reported hypercellular marrow, reduced number of megakaryocytes; presence of 31% of large-sized cells, large nucleus/cytoplasm ratio and hyperbasophilic cytoplasm with vacuoles-“starry-sky” appearance.

The histology describes as hypercellular with diffuse pattern lymphoid infiltrate, consisting of intermediate to large sized cells with irregular nuclei and evident nucleolus: CD20+ CD3- CD34- TdT- MPO- CD117- Cam5.2-.

Flow cytometry confirmed the presence of 21% of blasts with CD5- CD10+ CD19+ CD20- CD34- CD38+/++ CD43+ CD79b-/+weak CD200-/+weak cKappa+ TdT- immunophenotype. Hyperdiploid with a high proliferative index of 62%.

Cytogenetics identified the t(8;14)(q24;q32) involving the MYC and IGH genes.

The patient died at home while awaiting laboratory results.

Discussion: This case illustrates the important role of the clinical pathologist in orienting diagnosis. But, for establishing the diagnosis, a combination of different diagnostic techniques is necessary namely immunophenotyping, histology, cytogenetics and molecular biology.

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INFECTIVE ENDOCARDITIS BY ABIOTROPHIA DEFECTIVA: A CASE REPORT

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Abiotrophia defectiva is a fastidious gram-positive coccus which is part of the Nutritionally Variant Streptococci (NVS) group. It can be found in the flora of the mouth, gastrointestinal tract and urogenital tract. Abiotrophia defectiva has difficulty growing when subcultured if not supplemented with pyridoxine. Rarely it can be a cause of infective endocarditis.

This case describes an 85-year-old man sent to the emergency department after blood work in a telemedicine appointment revealed anaemia. The patient presented with fever of unknown origin. Relevant medical history included a prosthetic aortic valve, type II Diabetes mellitus and a recent ischemic stroke.

Blood and urine cultures were made. A gram-positive coccus was detected in all three blood cultures. Identification was done with an automated Vitek 2 Compact device, which yielded a presumptive diagnosis of Abiotrophia defectiva. This result was confirmed by an external laboratory.

Antibiotic sensitivity testing was done using E-test stripes on a petri dish, revealing that this strain was sensitive to penicillin, ceftriaxone, vancomycin and gentamycin.

Treatment with Ceftriaxone and Gentamycin had already been initiated and was maintained until 42 days of antimicrobial treatment was completed. During this period the patient had a favourable outcome with no complications.

Sometimes it is not possible to identify Abiotrophia defectiva with automated and semi-automated devices routinely used in the laboratory. As such, the microbiologist must take this uncommon diagnostic into consideration, as well as the alternative techniques which may be necessary for its identification and antibiotic sensitivity testing. Late diagnosis and treatment of Abiotrophia defectiva induced infective endocarditis (IE) is associated with poor prognosis when compared with IE of more common etiology.

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FOSFOMYCIN SUSCEPTIBILITY USING VITEK®2 AST-355 AND DISK DIFFUSION METHOD

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Introduction: Several in vitro studies have demonstrated that fosfomycin has an excellent activity against Escherichia coli (E. coli), including extended spectrum beta-lactamases (ESBLs), isolated from patients with urinary tract infections (ITUs). Vitek®2 AST-355 card is an usual method to determine fosfomycin susceptibility to Escherichia coli, presenting breakpoints of S≤16 and R>16 mg/L. Recently the European Committee on Antimicrobial Susceptibility Testing (EUCAST) has defined a Minimal Inhibitory Concentration (MIC) breakpoints for oral fosfomycin in E. coli of S≤8 and R>8 mg/L, which raises some doubts on fosfomycin susceptibility interpretation.

Objective: The aim of this study is to evaluate if the results of fosfomycin susceptibility using Vitek®2 AST-355 continue to be suitable for it´s use in routine laboratory susceptibility testing without the need to be verified by another method.

Methods: A prospective study using E. coli community strains (n=1951) isolated from urine samples of two laboratories were tested for fosfomycin susceptibility. The chosen methods were Vitek®2 AST-355 card and disk diffusion method (R≤24, S>24 mm), according to EUCAST recommendations. Discrepancy between results were confirmed using fosfomycin MIC Strip. All methods were equally and strictly used in both laboratories.

Results: Overall, of 1951 patients, 1651 (84,6%) were female (major prevalence over the age 80-89 years) and 300 (15,4%) were male (major prevalence 70-79 years of age).

Of the 1951 E. coli strains tested 4.5% were ESBL producers, 99.4% (n=1939) showed good agreement between the two methods. Discrepant results were obtained in 0.62% (n=12). Of these, 4 were resistant by Vitek®2 AST-355 and were confirmed by E-test. The remaining 8 were susceptible to fosfomycin by Vitek®2 AST-355 and resistant by E-test, corresponding to 0.41% of discordant results. In this study Vitek®2 AST-355 method had a sensitivity of 99.6% and a specificity of 100%.

Conclusions: In this study, a very good agreement was obtained between the two methods (99.38%). Despite the limitation of Vitek®2 breakpoints, with a MIC higher than the one recommended by EUCAST, these results show evidence that Vitek®2 AST-355 is an acceptable option to use in laboratory routine when determining susceptibility of E. coli to fosfomycin.

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CONTAMINATION RATE OF MGIT 960 CULTURES AND RECOVERY FROM REPROCESSED SPECIMENS IN A TERTIARY CENTRE, 2017-2020

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Introduction: Mycobacteria Growth Indicator Tube (MGIT) 960 liquid system is widely accepted as the gold standard for faster tuberculosis diagnosis, with an average time to detection of 12-16 days. However, it is easily contaminated with normal flora, leading to further reprocessing for optimal recovery. Strategies to control contamination include increase the volume of inhibitors and NALC-NaOH and an extended time of sample digestion. Contamination rate and recovery monitoring are recommended as quality indicators of laboratory detection and identification of mycobacteria.

Objectives: To report MGIT culture contamination rate and recovery rate of mycobacteria after reprocessing between 2017 and 2020, at Centro Hospitalar Tondela-Viseu.

Material and Methods: A retrospective analysis was performed on every MGIT culture. Contamination rate was assessed by the presence of normal flora in acid-fast smears from MGIT cultures flagged as positive. Recovery rate was calculated only in reprocessed specimens [MGIT + Löwenstein-Jensen (L-J)], in which acid-fast bacilli was detected in ZN smears and further identified, either by Ag MPT64 or molecular methods.

Results: 3452 specimens were processed, with an overall culture contamination rate of 11.6% (17.6%, 11.6%, 9.7% and 7.8% from 2017 to 2020 respectively). The most frequent contaminated specimens were sputum (46.6%), bronchial aspirates (38.6%) and tissues biopsies (13%). From 401 reprocessed specimens, the overall recovery rate of mycobacteria was 18.5% (74/401), being 22%, 16%, 14% and 20% from 2017 to 2020 respectively. Recovery efficiency was higher with L-J (17.2%), when compared to the second MGIT culture (10.7%). The simultaneous recovery with both media was achieved in 38 specimens (9.5%). The second MGIT tube was contaminated in 30 positive L-J cultures.

Conclusions: We observed consistent decrease trend in the contamination rate (56% change), which can be explained by improved adherence to best practices. The recovery rate from reprocessed specimens remained constant, which is a good indicator of a stable process. In our study, MGIT 960 has had a smaller rate of recovered mycobacteria than L-J. The main reason could be the higher rate of contamination. Our results highlight the importance of monitoring these quality indicators and the added value of L-J media in recovery of mycobacteria from reprocessed MGIT 960 cultures.

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MICROSPORUM CANIS INFECTION IN A PAEDIATRIC PATIENT: A CASE REPORT

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Keywords: Microsporum canis; dermatophytes; paediatric

Introduction: Microsporum canis is a zoophilic dermatophyte with cats and dogs as natural hosts. M. canis is highly contagious, easily transmitted to humans, causing glabrous skin (tinea corporis) and head (tinea capitis) infections. Tinea capitis (TC) is a common cutaneous fungal infection among 2 to 7 year old children but rare in the first year of life.

In this present case, cultures were performed using SGC2 agar to identify dermatophytes. Plates were incubated at 25 °C and examined every 2–3 days along 15 days. Identification was based on macroscopic and microscopic observation. Macroscopic examination revealed some white fluffy spreading colonies with a characteristic deep yellow-orange pigment reverse. “Spindle” shaped multicellular macroconidia with thick cell walls were observed on microscopic examination. Clinical features and culture results revealed a M. canis TC.

Case presentation: We present a case of a 2-year-old and a 1-year-old siblings presenting erythematous scalp lesions combined with 20 cm diameter extensive alopecia. Suspecting dermatophytosis a mycological analysis of all lesions was performed. Clinical features, direct examination, and culture results confirmed tinea capitis caused by M. canis.

Itraconazole (an imidazole/triazole type) has been the antifungal treatment of choice.

Discussion/Conclusions: In children, TC is sometimes misdiagnosed and underreported because of its similarity to other scalp pathologies. Therefore if erythematous scalp lesions are present those must be examined minding a probable fungal infection. Once diagnosed, TC treatment can pose a dilemma because different factors (i.e. safety, age, formulation, cost) may influence choice among equally effective therapies. This case report suggests that it is important to establish an accurate diagnosis and treatment to avoid recurrences or therapeutic failures, especially in children.

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STUDY OF SARS-COV-2 VARIANTS OF CONCERN BY REAL TIME REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION BETWEEN APRIL AND JUNE 2021

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Background: Alpha was, at the time, Delta, Beta, Gamma and Omicron are variants of concern (VOC) that present mutations in the spike protein. For these variants, evidence demonstrates a meaningful impact on transmissibility, severity, therapeutic decision and/or vaccine immunity. VOC monitoring is important to manage local public health measures and control local transmission chains.

Objectives: Identify circulating VOC by real-time reverse transcription-polymerase chain (RT-PCR) in naso/oropharyngeal SARS-CoV-2 positive samples from April to June 2021.

Material and methods: We used two real time RT-PCR assays. Positive samples were chosen randomly: 214 were analyzed between April and May by Assay 1; and 105 in June by Assay 2. Assay 1 allowed the qualitative detection of SARS-CoV-2 spike mutations N501Y (Beta/Gamma/Alpha variants), E484K (Beta/Gamma variants), HV 69/70 del (Alpha variant). Assay 1 did not distinguish between Beta and Gamma variants. Assay 2 allowed the qualitative detection of SARS-CoV-2 spike mutations L452R (Delta variant), K417T (Gamma variant), and K417N (Beta variant).

Results: SARS-CoV-2 had been detected on 184/12082 suspected samples (1,52%) analyzed in April, on 266/13400 samples (1.99%) in May and on 663/13948 samples (4.75%) in June 2021. Selected positive samples included 146 females and 163 males, aged 14 days to 94 years old. Assay 1 detected with high predictive value: 78 Alpha variants, 26 Beta or Gamma variants. No mutations or variants were identified in 110 samples, 54 (49,1%) in the second half of May. Assay 2 detected with high predictive value: 95 Delta variants, 4 Delta with K417N mutation (AY.1 sub-lineage) and 2 Beta. No mutation or variant were identified in 4 samples.

Conclusions: RT-PCR was a rapid approach to SARS-CoV-2 variants detection. Its results should be confirmed by sequencing. A change in the variant's predominance occurred in June. Simultaneously, more real time RT-PCR tests for SARS-CoV-2 identification were done and more positive samples were detected in June, coincident with the emergence of VOC Delta, than in May or April when VOC Alpha was predominant. It corroborates the data of the program of SARS-CoV-2 genomic surveillance published by the Portuguese National Institute of Health, at the same period.

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NOCARDIA BRASILIENSIS ISOLATED IN AN ELBOW WOUND ABSCESS

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Introduction: Nocardiosis is an infection caused by microorganisms of Nocardia spp. Nocardia brasiliensis, belonging to Nocardia spp., is a gram positive bacillus, aerobic, partially acid-alcohol resistant, filamentous and branched, with predominantly fastidious growth.Nocardia spp. can colonize the skin and respiratory tract, being an opportunistic agent that, in immunocompromised patients or with disabling pathologies, is responsible for causing localized or disseminated disease. The microorganism can also be introduced into the body due to traumatic causes, by direct inoculation of microorganisms. The standard method for diagnosing Nocardia spp. infection is isolation on cultural examination.Nocardia brasiliensis is not part of the nocardia-asteroid complex of antimicrobial susceptibility.

Clinical case: We present a clinical case of an 82-year-old female patient, non-insulin dependent diabetic, followed up at the Health Center due to a blunt wound in her left elbow, with a month of evolution, as a result of a fall from her own height. During this period, she did flucloxacillin without resolution of the clinical picture, but with improvement. The wound was characterized by inflammatory signs associated with a local abscess approximately 5 cm deep and with spontaneous purulent drainage.Due to the maintenance of her clinical condition, the patient was referred to the Emergency Department, where she was observed. In the emergency department, superficial exudate from the skin and soft tissues was collected - by swab - for microbiological study, and the patient was immediately discharged, with an empirical prescription of amoxicillin and clavulanic acid. In the microbiological exam, Nocardia brasiliensis was isolated.

Discussion and Conclusion: The case presented illustrates the importance of alerting to the possibility of infection by microorganisms that, although not common, have important repercussions. Cases of chronic infection may be based on less common fastidious microorganisms, and their correct identification is important so that the therapy can be properly directed, in order to avoid the perpetuation of the infection.

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BCGITIS IN AN END STAGE RENAL DISEASE PATIENT

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Introduction: Bacillus Calmette-Guérin (BCG) is a strain of Mycobacterium bovis that is included in the Mycobacterium tuberculosis complex (MTC). BCG was initially developed as a vaccine to prevent tuberculosis. However, since 1977, it has been used as intravesical immunotherapy for treatment of non-muscle invasive bladder cancer. BCG instillations are usually well tolerated, although systemic and local infections can occur in 1 to 5 percent of patients.

Case report: A 74 year old male with multiple comorbidities, including stage 5 Chronic Kidney Disease (CKD) and high risk non-muscle invasive bladder cancer treated with BCG intravesical immunotherapy, presented to the ER with generalized pain, foul smelling urinary discharge and urgency (patient was usually anuric) and fever. The type II urine test revealed leukoerythrocyturia, resulting in a diagnosis of a urinary tract infection (UTI) and the patient being medicated with cefixime.

A week later, the patient was returned to the ER, after a hemodialysis session, in a confused state, maintaining urinary symptoms and fever. Inflammatory markers were elevated and type II urine test was unable to be performed due to macroscopic pyuria. The diagnosis of a cefixime resistant UTI was assumed, the antibiotic was changed to piperacillin/tazobactam and the patient was admitted to an Internal Medicine Ward. Urine and blood cultures were negative. 3 days later, after absence of improvement, micobacteriological urine smear and culture were ordered. The smear showed numerous Acid-Fast Bacilli (AFB) and the culture was positive for cord forming AFB, which were identified by GenoType MTBC kit to be BCG. The patient was diagnosed with bladder BCGitis and was transferred to the Infectious Diseases Ward were he was treated with a HRE regimen and showed symptomatic improvement.

Discussion: This case report aims to demonstrate the necessity of a careful evaluation of a patient's medical history before ordering a microbiological exam. The treatment with intravesical BCG should have raised suspicion of a BCG infection. Moreover, in patients with no urinary excretion, the insufficient BCG clearance could lead to its incidental detection. However, the patient's systemic and local symptoms suggested actual BCG infection.

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CLINICAL INFORMATION: THE MISSING PIECE TO URINE CULTURE PUZZLE?

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Introduction: Urine culture plays a key role in the management of Urinary Tract Infections (UTI). Correct interpretation of urine culture results requires communication between clinicians and laboratory (lab). If possible, the clinician should provide the lab with enough clinical information to allow determining what colony count distinguishes true infection from contamination. Studies have proposed the use of different cutoffs in colony counts based on clinical presentation. Important information includes patient data (age and sex); general clinical information (symptoms, diagnostics and antibiotic therapy) and method of collection of urine submitted (voided or straight catheterization). However, microbiology labs frequently receive little or no clinical information about patients.

The aim of this work was to assess the clinical information in urine cultures' requisitions during 2021 in one hospital.

Statistics: 12463 urine cultures were processed in this lab in 2021 (a 30.2% increase from 2020). They represented the most frequent test in the microbiology department (21.9% of all tests). Patient age and sex were present in all urine culture requisitions (average age was 55.2 years and 62.5% of patients were female). In spite of being a mandatory field when requesting urine culture, "General Clinical Information" was completely absent in 24.9% of requisitions (text box being filled with a dot or a single letter). Furthermore, in 14.6% of requisitions the only clinical information present was the acronym "UTI". Intensive Care Department provided information in 100% of requisitions. On the other end of the scale, less than half of Emergency Department requisitions had clinical information (44.7%). Information regarding the collecting method of urine submitted to the laboratory (not a mandatory field) was only stated in 29.8% of requisitions

Discussion: In this study, urine culture was found to be requested in large scale. The necessary information was frequently absent from requisitions. We did not find similar studies to compare data. This work highlights the need to improve communication between the lab and clinicians. Strategies may involve restructuring the urine culture requisition form and raising awareness to the importance of clinical information in the quality of urine culture results.

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A COMPARATIVE STUDY ON THE PERFORMANCE OF STANDARDTM M10 FOR THE DIAGNOSIS OF SARS-COV-2 INFECTION

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Background: The SARS-CoV-2 pandemic has become a challenge for everyone, particularly the Clinical Pathology department, where it stimulated the development of innovative techniques that improved the quality and response time. The STANDARDTM M10 SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of RNA from SARS-CoV-2 using reverse transcription (RT) real-time PCR. It integrates sample preparation, nucleic acid extraction and amplification, and detection of target sequences on E and ORF1ab genes, in approximately 1 hour. The GeneXpert® (Xpert Xpress) assay is an FDA-authorized RT-PCR assay used for the diagnosis of SARS-CoV2 infection and detects E and N genes of the novel coronavirus, also in nasopharyngeal or oropharyngeal swab specimens.

Objective: To evaluate diagnostic sensitivity and specificity of the STANDARDTM M10 SARS-CoV-2 by comparing with GeneXpert® (Xpert Xpress) in routine clinical practice.

Methods: A retrospective, single institute, randomized study was conducted. Tests were performed with specimens obtained in the hospital during routine clinical practice. All samples were fresh and the same swabs, properly placed in viral transport medium (VTM), were used for both devices. The procedure was done according to the instructions for use of STANDARDTM M10 SARS-CoV-2 and GeneXpert® (Xpert Xpress). The RT-PCR results of those samples were not blinded to the laboratory staff. The performance and concordance rates between STANDARDTM M10 and GeneXpert® SARS-CoV-2 assay, regarding the detection of SARS-CoV2 E gene, were evaluated.

Results: 138 samples were studied, of which 56 were positive and 60 were negative for E gene. STANDARDTM M10 SARS-CoV-2, showed a high level of sensitivity (98,1%) and specificity (96,6%) as compared to the evaluation criteria. Due to the lack of sample volume for re-testing of invalid results, 22 specimens (15,9%) were excluded from the experiment (drop-out); GeneXpert® had 5 samples (3,6%) re-tested due to invalid results.

Conclusions: STANDARDTM M10 SARS-CoV-2 is a potential useful diagnostic tool to accurately test for the novel coronavirus infection. The percentage of invalid results is probably related to the first version of the software. Currently, the M10 has a new software version that was not possible to test in this study.

|P72

URINARY TRACT INFECTION IN A COMMUNITY LABORATORY

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Goal: Study the prevalence, etiology and antibiotic susceptibility profiles of the main agents responsible for urinary tract infections (UTI) in 2021 in a community laboratory.

Materials and Methods: 4019 urines were analyzed. Positive urines (N = 1422) were evaluated for the etiologic agent and the susceptibility profile to antibiotics using the Vitek 2 Compact system (bioMérieux). Amoxicillin, amoxicillin/clavulanic acid, cefuroxime, cefotaxime, ertapenem, ciprofloxacin, fosfomycin, gentamicin, cotrimoxazole and nitrofurantoin were the antibiotics studied.

Results: The prevalence of UTI was 35.4% (14.1% in males and 85.9% in females). Escherichia coli (55.6%) was the predominant uropathogen isolated, followed by Klebsiella pneumoniae (14.6%), Proteus mirabilis (7.8%) and Enterococcus faecalis (4.6%). For Enterobacterales, the antibiotics with higher resistance were amoxicillin, amoxicillin/clavulanic acid, ciprofloxacin and cotrimoxazole. Fosfomycin showed a resistance rate of 55% for K. pneumoniae. E. coli presents high susceptibility to fosfomycin and nitrofurantoin, 97% and 99%, respectively. E. faecalis showed 2.0% resistance to amoxicillin and nitrofurantoin. Resistance to 3rd generation cephalosporins by the production of expanded-spectrum beta-lactamases (ESBL) was 33.0% and 8.0% for K. pneumoniae and E. coli, respectively. Regarding resistance to carbapenems, nine multi-resistant strains (K. pneumoniae producing carbapenemases-KPC) were detected.

Conclusions: E. coli was the major UTI pathogen. High degree of resistance to ciprofloxacin, amoxicillin, amoxicillin/clavulanic acid and cotrimoxazole, shows that the broad use of these drugs needs to be revised, demonstrating its low efficacy as empirical therapy. As documented in the present guidelines, nitrofurantoin and fosfomycin are a good therapeutical option for E. coli UTI, especially in ESBL producers. The high resistance found for the other bacterial strains reveals that caution should be exercised in the empirical prescription of these antibiotics since E. coli is responsible for only 56% of UTIs. Multidrug resistance due to the production of ESBL or carbapenemases is more frequent in K. pneumoniae, being an emerging public health problem both at the hospital and in the community.

|P73

LABORATORY TESTS FOR SARS-COV-2 SCREENING

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Background: COVID-19 is considerated an acute respiratory disease caused by the severe acute respiratory syndrome coronavirus 2(SARS-CoV-2). The disease pandemic continues to affect much of the world and it is important a clear understanding of the nature of the tests and the interpretation of their found because of the impact on public health. Several measures were adopted to contain the expansion of SARS-CoV-2 infection, including the definition and implementation of the national testing strategy for SARS-CoV-2.

Objectives: Recognize the diverse SARS-CoV-2 screening tests and the criterium for use and management.

Methods: Bibliographic revision of the literature available in: DGS, INFARMED, Portuguese National Institute of Health (INSA) and European Control of Diseases (ECDC).

Discussion: Screening tests available in Portugal, according to DGS, INFARMED and INSA are nucleic acid amplification molecular tests (NAAT) and Rapid Antigen tests (TRAg). NAAT is the gold standard for diagnosis and screening, include conventional real-time RT-PCR tests and rapid amplification tests nucleic acids. TRAg for professional use has ≥ 90% sensibility and ≥ 97% specificity compared with NAAT and should be used during the first 5 days of symptoms, in self-test mode presents ≥ 80% sensibility and ≥ 97% specificity compared with NAAT but have more risks of falses positive or negative results. Taking into account the performance and reliability characteristics of the tests ,the ones that provide the greatest guarantee in the protection of Public Health are TAAN and professional-useTRAg tests, according to the ECDC, the use of TRAg in the self-test mode may have an impact on transmission control when carried out by asymptomatic individuals before social events, , provided that there is a communication strategy and empowering citizens, as well as the implementation of control mechanisms to avoid the falsification of the results. Serological tests are not used for the diagnosis, it evaluates the immunological response.

Conclusion: Portuguese national strategy for the SARS-CoV-2 screening is adapted to the epidemiological evolution of the pandemic situation. It is important that you know the different tests and when to be carried out. Any doubtful result should be repeated using the gold standard test - NAAT.

|P74

TRANSMISSION OF EPSTEIN BARR VIRUS (EBV) DURING THE SARS-COV-2 PANDEMIC

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Introduction: EBV, human herpes virus type 4, belongs to the Herpesviridae family. It spreads primarily through saliva and infects and replicates in the oropharyngeal epithelium and salivary glands.

Objectives: Taking into account the same mode of transmission of EBV and SARS-Cov-2 viruses by airborne route and droplets, the authors intended to analyze retrospectively the evolution of EBV incidence during three autumn-winter periods 2019-2022.

Simultaneously, they want to evaluate the influence of the public health measures taken to counteract the SARS-Cov-2 pandemic on EBV transmission.

Materials and Methods: To accomplish this investigation, data from 696 patients were collected from Clinidata software and the following parameters were recorded: number of requests, age, functional unit where the requests were demanded and positivity for IgM. The positivity for IgM was measured by two methods: immunochromatographic test with heterophil antibodies of IgM class MNITOP®OPTIMA (manufacturer: BioSYNEX) and/or measurement of IgM by ELFA method (Enzyme Linked Fluorescent Assay) in the VIDAS® equipment (EBV VCA IgM – antibodies of class IgM to Viral Capsid Antigen) with Cut-off >0,18.

Results: Regarding the age group, the highest incidence was recorded in the group from 0 to 19 years old. A reduction of the total number of requests in autumn-winter 2020/2021 was found, although the percentage of positive results remained constant across the years, 9%-11%.

The majority of cases (53%-100%) was detected in the Emergency Department, during the three periods of the study.

Conclusion/Discussion: The reduction of total number the request in autumn-winter 2020/2021 can be due to the reduction of Emergency Department attendances during the third SARS-Cov-2 wave. On the other hand, the percentage of positive tests remain constant along the studied period, with a mean value of 10%.

Taking into account the common mode of transmission and the generalized use of facemasks and other preventive measures against SARS-Cov-2, EBV infection might have presented a decrease, yet this was not observed.

It is well known that the age range of greater EBV risk (0 to 19 years), and especially pre-school children, had less adherence to social distancing measures and to the use of masks. This might, at least, in part, explain the obtained results.

|P75

TRIS [2-CARBOXY-ETHYL] PHOSPHINE HYDROCHLORIDE AS A VITAMIN C STABILIZER, DOES IT WORK? MEASUREMENT BY LC-MS/MS

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[1]CHUC

Introduction: Vitamin C (VitC) or L-ascorbic acid is a water-soluble antioxidant vitamin obtained only through diet or supplementation.

Vitamin C is essential to many abolic pathways, is very unstable in vitro, rapidly oxidizing to dehydroascorbate. This oxidation process is due to pH, high temperature and presence of oxidizing enzymes or iron ions, decreasing the concentration of VitC.

Studies indicate that tris [2-carboxy-ethyl] phosphine hydrochloride (TCEP) can slow down this oxidation process. Pre-analytical stability assessment is essential to define maximum storage time while maintaining sample stability.

Aim: The study intends to evaluate the pre-analytical stability and the viability of using TCEP to define the flow of samples aiming at the good laboratory practices of the VitC assay.

Material and Methods: Ten blood samples were collected into lithium heparin tubes, protected from light and placed on ice. Centrifugation was performed at 4ºC. Aliquots were made, with and without stabilizer, and stored at -20°C.Five measurements were performed by Liquid Chromatography in Tandem Mass Spectrometry (LC-MS/MS) and quantified (mg/L) in a single analytical run. T0 on the day of collection, T1, T2, T3 and T4 at 3, 7, 14 and 21 days respectively.

Result bias (%) and absolute variation were calculated at different times.

Results: Mean bias variation of samples without stabilizer was: - 14.6% (T0-T1), -48.8%, (T0-T2), -77.5% (T0-T3), -85.1% (T0 -T4) that correspond an average decrease of 11.77 mg/l in the VitC sample.

Mean bias variation of samples with stabilizer was: -5.9% (T0-T1), -20.4%, (T0-T2), -24,5% (T0-T3), -28,6% (T0 -T4) that correspond an average decrease of 4.15 mg/l in the VitC sample.

Mean bias variation of intra-sample (with and without TCEP) in the same run was: -4.6% (T0), -11.9% (T1), -37.8% (T2), -72.2% (T3) and -80.5% (T4).

Conclusion: Despite the small sample of this study, we realized that the use of TCEP is essential to improve the laboratory pre-analitical time, to ensure the best practices for measuring Vit C.

Without TCEP, samples can be frozen for up to 7 days without clinical impact. Using the stabilizer this time increases up to 21 days with a bias of 28.5%, however acceptable in this methodology. The use of TCEP improves the laboratory pre-analytical workflow at least 21 days.

|P76

DIRECT AND INDIRECT ISE POTENTIOMETRY AND PSEUDOHYPONATREMIA - A CLINICAL CASE

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Introduction: Potentiometry by ion selective electrodes is the method of choice for measuring electrolytes concentration and is subdivided into indirect (IP) and direct potentiometry (DP). Both measure electrolyte concentration on the water phase, but the former requires a dilution of total serum, assuming a fixed solid phase proportion (around 7%, containing lipids and proteins). DP, used in blood gas analysers, measures on a whole blood sample without the need of dilution, and as such, not affected by variations of lipids and proteins.

Clinical case: A 66 year old male with known medical history of multiple myeloma presented to the emergency department with pain in the right thigh. He underwent radiography, which revealed a lytic lesion in the femoral neck. The initial study on a serum sample revealed total proteins of 123.5 g/L, sodium (Na^+) 132 mmol/L and negative lipemic index. Due to hyponatremia with elevation of total proteins, a new measurement on whole blood was performed in the gasometer which revealed Na^+ 142, mmol/L. The clinical team was notified and the latter Na^+ value was reported, as it was obtained with the most adequate methodology, in this case. The patient was admitted to Internal Medicine for symptomatic control, with no need of Na^+ correction.

Discussion: Pseudohyponatremia is a common laboratory abnormality defined by a Na^+ concentration lower than 135 mmol/L in the presence of an isoosmolality. Common causes include hyperlipidemic or hiperproteinemic conditions such as monoclonal gammopathy malignancies, amyloidosis and intravenous immunoglobulin therapies. It occurs in IP due to the dilution of the sample (total serum), which aims to reduce ionic interference. In this case, we observed a patient

with an increased total serum proteins (and as such, an increase in the solid phase). Since the dilution volume is fixed, it does not take in consideration changes in the water-solid phases proportions, so an increase in the solid phase leads to a falsely decreased Na^+ value. The measurement by DP allows a more accurate result as it does not suffer interference from lipids and proteins.

The perception by the Clinical Pathologist is crucial for the early detection of this error and avoiding undue treatment (as reported in literature1) and increased costs. Thus, laboratory communication with the clinical team is crucial for the correct diagnosis and treatment of the patient.

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|P77

WHAT IS THE IMPACT OF A BOOSTER DOSE OF A SARS-COV-2 MRNA VACCINE ON HUMORAL IMMUNITY? REAL DATA FROM A LARGE INDIVIDUALS COHORT

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Introduction: The SARS-CoV-2 pandemic was responsible for the death of millions of people around the world, which accelerated the study of vaccines. The BTN162b2 mRNA COVID-19 is a messenger RNA vaccine that encodes the spike protein of the virus. However, the duration of the protection conferred by this vaccine and factors associated with immune responses require validation in large cohorts.

Aim: Cohort observational study to assess the kinetics and factors predictive of humoral immune response to mRNA SARS-CoV-2 vaccine administration, in healthcare workers of a large tertiary university hospital center.

Material and Methods: In early 2021, 4509 healthcare workers completed vaccination. From these, 1673 (82.2 % Female) participated in this study and collected blood samples:

– T0 – 24h-48h before vaccination
– T1; T2 and T3 - 15, 90, 180 days after vaccination, respectively
– T4 - 24h-48h before 3th dose (10 months after 2nd dose of vaccine)
– T5 – 3 weeks after 3th dose

Peripheral blood was collected for immunological analysis using the Quant SARS-CoV-2 IgG II Chemiluminescent Microparticle Immunoassay (CMIA) to determine anti-spike IgG, receptor binding domain (RBD), S1 subunit of SARS-CoV-2 (IgG titer above reactivity cut off, 50 AU/mL).

Results: At T0, 100% (n=1673) of participants enrolled were naïve and had non-reactive IgG antibodies to SARS-CoV-2. Fifteen days after completing the vaccination, the IgG overall median titer was significantly elevated (21.3 x103 AU/ml). Comparing data with T1 results we observed a waning of antibody titers throught time: a decline of 6.6 fold (-84.8%) at T2; 20.36 fold (-95.1%) at T3; 36.1 fold (-97.2%) at T4. At T5, after 3th dose, a rise of 0.71 fold (+40.56%) compared with T1.

Conclusions: After vaccination with the BNT162b2 vaccine, anti-SARS-CoV-2 IgG kinetics tend to peak around 14 to 30 days, followed by a substantial reduction over time, with significantly lower levels at 10-months as described in similar studies. Also the rise of antibodies after booster, above those found in T1, is described elsewhere but the long-term durability of such protection remains to be determined.

These findings support the need to track humoral immunity kinetics to uncover viral susceptibility and eventually implement re-vaccination, particularly in groups prone to lower humoral immune response.

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A PRACTICAL APPROACH TO EVALUATE THE BEST ANALYTICAL LONG-TERM CV FOR RCV CALCULATION

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Introduction: Reference Change Value (RCV) is one of the main indicators of the clinical quality of a dosing method for personalized patient monitoring. Its calculation involves the use of intra-individual biological variation data and long-term analytical CV (CVa) (1,2). The highest CVa of the less precise internal quality control level can be used to calculate the RCV for all the different concentration or activity of a dosing method.

Depending on the method precision, we can have more than 30% of patient results with analytical errors beyond the highest CVa.

A practical way to estimate the percent number of patients results (PR) beyond the CVa is to use the laboratory information system (LIS) to calculate the percent of controls beyond the highest CVa (CTLcv).

If the PR beyond a CVa is unacceptable, we can calculate the PR under the error of the extended uncertainty measurement (2xCVa), that usually includes 100% of PR.

Material and Methods: We use the Modulab Gold LIS to evaluate the CTLcv of the last 6 months that have errors above and under +-1 sd for the highest CVa observed on the quality control charts for potassium (K), total cholesterol (Col) and thyroxine-free (FT4).

We calculate the RCV using the online calculator at EFLM Biological Variation Database (2), for 95% probability.

Results: The CTLcv estimated for the highest CVa were: K= 21.6%; Col=32.8% ; FT4= 23.8%

The RCV calculated for the highest CVa : RCVK= 11.8% ; RCVCol=15.4% ; RCVFT4= 24.5%

The RCV calculated for the expanded uncertainty of measurement (2xCVa) for the highest CVa and 0% CTLcv: RCVK= 12.9% ; RCVCol=17.3% ; RCVFT4= 43.1%.

Conclusion: We can use RCVK=11.8% for only 78.4% (100-21.6) of all of K results for monitoring patients, but with RCVK=12.9% we can use it for monitoring all of K patient results.

We can use RCVCol=15.4% for only 67.2% (100-32.8) of all of Col results for monitoring patients, but with RCVK=17.3% we can use it for monitoring all of Col patient results.

We can use RCVFT4=24.5% for only 76.2% (100-23.8) of all of K results for monitoring patients, but with RCVK=43.1% we can use it for monitoring all of FT4 patient results.

When we use CVa that CTLcv=0%, on RCV calculations, it include the analytical error of all measurements and therefore adequate for monitoring all patients.

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AN UNEXPECTED CAUSE OF HYPERTHYROIDISM

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Introduction: Hyperthyroidism has different etiologies. Graves' disease (GD) is an autoimmune disorder caused by antibodies targeting the thyrotropin receptor of the thyroid cells (TRAb), leading to an increased production of thyroid hormones and inducing an inflammatory process in the thyroid. Endocrine orbitopathy is often present in GD and some patients develop exophthalmos. In patients with GD orbitopathy there may be additional antibodies directed against the insulin-like growth factor type 1 receptor (IGF-1R) which is overexpressed on the orbital fibroblast. Another aetiology for hyperthyroidism is toxic adenoma, a single hyperactive autonomously functioning thyroid nodule that produces supraphysiological amounts of T4 and/or T3 resulting in suppression of serum thyroid stimulating hormone (TSH). The coexistence of GD and thyroid functioning nodules is rare and is called Marine-Lenhart syndrome, estimated to occur in 0.8-2.7% of patients with Graves' disease.

Case Presentation: We present the case of a 53 year old female referred for abnormal thyroid function test. Patient reported hair loss, palpitations and fatigue but denied any difficulty swallowing, bowel habits, weight loss, radiation to neck, new medications or family history of thyroid disease. Laboratory tests showed TSH <0.01 (0.27 - 4.2 uIU/mL), free T3 15.12 (2.57 - 4.43 pg/mL), free T4 3.5 (0.93 - 1.7 ng/dL) and anti-TSH of 27,0 IU/L (<1.75 IU/L). Thyroid ultrasound showed a right thyroid lobe nodule measuring 44 mm. Thyroid scintigraphy showed an intranodular distribution of 99mTc-pertechnatate consisting of an autonomously functioning thyroid nodule in the right lobe of the thyroid. The patient was treated with methimazole followed by thyroidectomy and four years after initial diagnosis TRAb´s were still present and patient had to undergo bilateral orbital decompression followed by bilateral blepharoplasty.

Discussion: Marine-Lenhart syndrome is a rare cause of hyperthyroidism with diagnosis criteria still not well established. Although hyperthyroidim caused by two different processes occurring in the same patient is a rare condition, it is important to have a high índex of suspicion when evaluating thyroid disease and perform complete thyroid function tests.

|P80

A LABORATORY LOOK IN TO WILSON´S DISEASE: A TEN YEARS RETROSPECTIVE STUDY

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Introduction: Wilson's disease is a rare and progressive autosomal recessive disorder in which changes in copper (Cu) metabolism result from the presence of mutations in the ATP7B gene. These changes lead to Cu deposition in different organs and clinical manifestations are directly related to the sites of excessive Cu accumulation, being liver failure and dysfunction of central nervous system the most frequent findings. Early diagnosis, initiation of therapy and monitoring of urinary (U) and serum (S) Cu levels are essential.

Objective: Evaluation of laboratory parameters of individuals with Wilson's disease, in a tertiary hospital.

Methods: Retrospective study of 25 patients diagnosed with Wilson's disease, for 10 years. Parameterized research using the laboratory's computer system to analyze the CuU, CuS and ceruloplasmin (Ce) values of these patients. Assessment and study of the following variables: age, gender, clinical presentation, laboratory tests at diagnosis, liver biopsies, rhodamine staining, liver Cu assay and presence of Kayser-Fleischer rings.

Results: The age of the patients ranged from 11 years to 69 years (33.6 ± 16.2 years), 5 in pediatric age, being 13 males and 12 females. Three (12%) had a family history of Wilson's Disease, 6 (24%) had no family history and 16 (64%) had no clinical information available. Cu in liver biopsy was determined in two patients, with an average concentration of 1018.5± ug/g. At the time of diagnosis, the mean CuS was 0.048±0.606mg/L, the CuU measurement was 0.471 ±0.298, and the Ce was

0.075±0.058g/L. Rhodamine staining was negative for all patients. In 28% of the cases, liver cirrhosis was the presentation of the disease, with ascites being present in 4% of the total. Only 4% had Kayser-Fleischer rings. From the genetic study carried out, it was found that 48% had mutations in the ATP7B gene, of which 20% had variants in homozygosity and 28% in heterozygosity.

Conclusion: The patients presented a significant reduction in Ce and in CuS and an increase in the CuU, with liver cirrhosis being the most frequent presentation. Avoiding deposition of Cu in tissues is fundamental in this life treatment disease, showing that laboratory determination of CuU/S and Ce is crucial to monitoring the adequacy of therapy.

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COMPARATIVE STUDY OF TWO AUTOMATED IMMUNOASSAYS FOR THE DETERMINATION OF NEURON-SPECIFIC ENOLASE

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Introduction: Neuron-Specific Enolase (NSE) is a cell-specific isoenzyme of enolase found in neuronal and neuroendocrine tissues. As a tumor marker, it can help support the diagnosis of small cell lung cancer, contributing to its monitoring and prognostic stratification, as well as for other neoplastic and nonmalignant diseases.

Nowadays, automation is fundamental to optimize the management of workflow and resources. When choosing an immunoassay, quality of the results is of paramount importance.

Objective: Evaluate the performance of two immunoassays to determine the concentration of NSE in serum samples.

Materials and methods: Over 5 months, 163 samples were sequentially processed in KRYPTOR® compact PLUS (BRAHMS®) (KB) – TRACE method – and in Alinity i® (Abbott®) (AA) – immunoassay microparticles by chemiluminescence; both according to manufacturer's instructions. Hemolyzed samples were excluded.

Manufacturer's reference values (RV) were: <12,7 ng/mL (KB) and <11,1 ng/mL (AA). Statistical analysis was performed using Microsoft Excel® software, including the determination of Pearson's correlation coefficient.

Results: Concentration ranges varied between 6,19–69,97 ng/mL (KB) and 4,0–57,9 ng/mL (AA).

Comparison between the two methods showed a strong positive correlation (y=0,9615x-3,9471; R2=0,94). The mean concentration of NSE was 16,68 ± 8,8 ng/mL (KB) and 12,10 ± 8,8 ng/mL (AA) (mean ± standard deviation (SD)). The mean difference between pairs of results was 4,59 ± 2,2 ng/mL. The mean bias was -30,67% with a variability of 12,7%.

With respect to samples exceeding RV, 114 samples were above RV after being analyzed in KB contrasting with 62 samples in AA, corresponding to 31,9% of potential discrepant clinical decisions, with the following means and SD: 19,5 ± 9,1 ng/mL (KB) and 18,4 ± 10,8 ng/mL (AA). Of notice, the mean difference between pairs of results was of 4,72 ng/mL (minimum 0.58 ng/mL, maximum 12,07 ng/mL).

Conclusion: The concentration of NSE may support diagnosis, monitoring and prognosis of several diseases.

The results obtained through the two methods revealed a good correlation, so both could be valuable options for laboratory automation for NSE determination.

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IMMUNE RESPONSE TO MRNA COVID-19 VACCINATION – A LONG TERM STUDY IN HEALTH CARE PROFESSIONALS

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In December 2019, SARS-CoV-2 was identified in city of Wuhan, China. It is a causative agent of an acute respiratory disease, coronavirus disease 20191. Vaccines to prevent SARS-CoV-2 infection are considered the most promising approach for curbing the pandemic and the global strategy to control COVID-192. SARS-CoV-2 antibodies are less useful in treating the

disease but will play an important role in determining and monitoring the immunity levels as the epidemic progresses3. The information provided by systematic hospital-based screenings can contribute to a knowledge gap on SARS-CoV-2 circulation in the general population4,5.

Our aim was evaluating the SARS-CoV-2 seroprevalence in our Hospital Center healthcare professionals (HCP), after Pfizer-BioNTech COVID-19 vaccination.

The Administrative Council approved a prospective study to evaluate the immune response after vaccine intake among HCP. A total of 1296 individuals (1011 female and 285 male) who were vaccinated and met all the criteria for this study, (absence of IgG (anti-RBD/S) antibodies prior to vaccination and monitoring of levels of each during approximately ten months) were selected. A representative sample of the data collected, was chosen and 440 CHTMAD HCP (340 females and 100 male), blood samples, that had been harvested before vaccination (T0) and after one (T1), three (T2), six (T3) and nine (T4) months after second dose vaccine uptake were analysed. The levels of antibodies were determined by a chemiluminescent microparticle immunoassay (AdviseDx SARS-CoV-2 IgG II, Abbott Diagnostics, Abbott Park, IL, USA), designed to detect IgG antibodies to the receptor binding domain (RBD) of the S1 subunit of the spike proteins of SARS-CoV-2. Results were analysed using GraphPad prism Software 6.0 and t-student test for statistical analysis.

The level of IgG (anti-RBD/S) antibodies decreased significantly over time ($p < 0{,}0001$), and no significant differences in antibody titers were observed by gender. When we compare the level of antibodies we could see the levels decreasing as the age increases (female: slope -197.5 to 54.05; male: slope -279.8 to 2.64). The anti-spike IgG level was substantially lower nine months after vaccination (T4). This data supports the need of a third dose of mRNA vaccine.

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|P83

EVALUATION OF TWO AUTOMATED IMMUNOASSAYS FOR THYROGLOBULIN ASSAY: ARE ALL ASSAYS CREATED EQUAL?

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Introduction: Thyroglobulin (Tg), synthesized in thyroid follicular cells, is the most expressed protein in the thyroid gland. The main application of this tumor marker is postoperative follow-up of patients with differentiated thyroid carcinoma, however it can also help in the diagnosis of Hashimoto's disease, Graves' disease, thyroid adenoma and carcinoma, amongst other diseases.

Tg determination is generally performed through immunoassays (IA). As multiple automation options are available, choice must take into account quality of the results, the target population and workflow of the laboratory.

Objective: This study aims to compare two IA to determine the concentration of Tg in serum samples.

Materials and methods: Over 5 months, 171 samples were processed sequentially in Cobas® e411 (Roche®) (CR) – electrochemiluminescence – and Alinity i® (Abbott®) (AA) – chemiluminescence microparticle immunoassay; both according to manufacturer's instructions.

Manufacturer's reference intervals (RI) were: 3,50-77,00 ng/mL (CR) and 3,68-64,15 ng/mL (AA). The lower limit of detection (LoD) were 0,04 ng/mL and 0,09 ng/mL for CR and AA respectively. Statistical analysis was performed using the software Microsoft Excel®, including the determination of the Pearson correlation coefficient.

Results: Concentration ranges varied between 0,04–341,90 ng/mL (KB) and 0,09–378,92 ng/mL (AA).

The two IA showed a strong positive correlation (y=1,0962x + 0,3867; R2=0,9957). Mean Tg concentration was 14,22 ng/mL (CR) and 15,98 ng/mL (AA). Mean difference between the results pairs was -1,75 ± 5,3 ng/mL.

Considering the RI, 124 (72,5%) samples showed Tg concentrations outside the RI in the CR, while in the AA there were 122 (71,3%), demonstrating 1,2% of potential discrepancy in the clinical decision.

Conclusion: Tg concentrations are essential in the diagnosis and follow-up of thyroid disorders.

The results of the two methods revealed an excellent correlation, with only 1,2% of discrepancy in clinical decision. The LoD differs between the equipments, the impact of which should be evaluated for the thyroidectomized patients. Our study showed that both the IA could be valid automated alternatives for clinical laboratories.

|P84

APPLICATION OF BIO-RAD D-100™ SYSTEM ANALYSER FOR HBA1C MEASUREMENT AND THE HEMOGLOBIN VARIANTS SCREENING

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Introdution: Diabetes mellitus is characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy, and affects approximately 7% of the world's population. HbA1c is the stable glucose adduct to the N-terminal group of the beta-chain of HbAo. The results of HbA1c can be used either for diagnosis or monitoring the glycaemic state in diabetic patients, with a frequency of two to three months interval, in human blood. The Bio-Rad D-100™ system is a fully automatic benchtop analyzer for determination of HbA1c, based on high performance liquid chromatography (HPLC), this equipment detects normal and abnormal peaks, such as hemoglobin variants. When a possible variant is detected, we reprocess the sample in the SEBIA MINICAP FLEX PIERCING equipment (Capillary electrophoresis), for confirmation. Analytical Interferences in HbA1c assays: red blood cell half-life; carbamylated hemoglobin; acetylated hemoglobin; Heterozygous hemoglobinopathies (variants S, F, C); Homozygous hemoglobinopathies (absence of HbA).

Aim: Evaluation of HbA1c values from a pool of samples, using Bio-Rad D-100™ system and the casuistic of the hemoglobin variants detected.

Materials and Methods: A retrospective study of HbA1c assays performed in the Bio-Rad D-100™ equipment and statistical evaluation of the presence of variants, after confirmation in the SEBIA capillary electrophoresis equipment. Data were analyzed using Microsoft Excel software.

Results: retrospective study of 13604 random samples, where the average age was 55.9 (± 17.4). For 6 months, the HbA1c value was calculated in all samples using the Bio-Rad D-100™ system and from these, 45 (0.33%) hemoglobin variants were found. HbS was detected in 32 samples (71.1%) and also the other variants such as HbF, HbC and HbE. These 45 hemoglobin variants found by HPLC were confirmed using the capillary electrophoresis method.

Conclusions: In conclusion, there is a very good agreement between the different HbA1c methods, for the HbA1c measurement. Direct detection by HPLC provides a relative and accurate quantification of the hemoglobin A1c fraction. All laboratories should have other equipments with other methodologies to confirm HA1c values in the presence of hemoglobin variants and to confirm and classified the variants themselves.

|P85

ATIVE B12 AND METHYMALONIC ACID IN THE ACESSEMENT OF VITAMIN B12 DEFICIENCY

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Introduction: Neurological and hematological disturbs can occur with vitamin B12 (B12) deficiency. This situation emerges when VitB12 is not absorb in the gastrointestinal track, including intestinal disease, pernicious anaemia and aging, or when dietary intake is not enough, as in vegan diets. B12 bonds mainly to two plasmatic proteins but the biologically available form is the one bonded to transcobalamine and represents 10-30% of the circulating B12. This form is called holotranscobalamine or active B12 (aB12) and some studies suggest that is more sensitive in the diagnosis of B12 deficiency. In cells, the lack of B12 stops some metabolic pathways and increases blood MMA and HCY.

It is often assumed that laboratory assay for serum B12 can test for the deficiency but the active form is more targeted and the functional assays, that include methylmalonic acid (MMA) and homocysteine (HCY) are important for B12 deficiency confirmation.

Sequential assays algorithms with combination of multiple markers have been proposed and can be the key to improved diagnosis.

Aim: The aim is to evaluate the assays B12, aB12, MMA and HCY in the evaluation of B12 deficiency.

Material and methods: One-year retrospective study with, at least, two of the following patient results: B12, aB12, MMA or HCY. Cutoff values were defined as: <250pg/ml for B12 (considering elderly and pediatrics); <70pmol/L (possible deficiency) and <25pmol/L (deficiency) for aB12; >0,400µmol/L for MMA and 15,0µmol/L for HCY.

Results: A total of 170 patients were selected being 156 with B12 (23 were <250pg/ml), 42 with aB12 (26 were <75pmol/L), 158 with MMA (29 were >0,400µmol/L) and 78 with HCY (19 were >15,0µmol/L). Samples with B12 <250pg/ml revealed 57,7% with MMA or HCY above cut-off and 39,1% with aB12 <75pmol/L. Samples with aB12 <75pmol/L revealed 34,6% with MMA or HCY above cut-off and all samples with aB12 <25pmol/L had MMA >0,400µmol/L. An equal number of samples (9) were found with B12 <250pg/ml and aB12 <75pmol/L and with aB12 <75pmol/L and positive functional markers for B12 deficiency.

Conclusions: the majority of samples with B12 <250pg/ml had at least one functional marker positive for B12 deficiency suggesting that additional studies have to be performed in these patients. aB12 revealed to be a good assay for clinical algorithms and to confirm vitamin B12 deficiency.

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|P86

THE USE OF PROCALCITONIN DURING THE COVID-19 PANDEMIC: SINGLE-CENTRE RESTROSPECTIVE AUDIT

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Procalcitonin (PCT) has been widely studied as a biomarker for early detection of infection, to predict mortality and guide antibiotic management. The COVID-19 Pandemic was a trigger to initiate PCT measuring at our hospital. Our goal was to audit the use and performance of PCT as a biomarker of infection.

PCT was measured 10 387 times in 3690 adult patients between 01-04-2020 and 31-12-2021. Patients were mostly men (62%) from the 70-79 age-group. PCT measurement was mostly used by Intensive Care Units (51%) and in COVID-19 patients (83%), with an overall mean of three determinations per patient. The mean value of PCT was 2,22ng/mL, with 30% values being above reference (>0,5ng/mL), of which 66% were from ICU patients. In order to evaluate PCT performance as a biomarker for infection we restricted our search to PCT values obtained from patients in ICU between 01-01-2021 and 31-03-2021, with

C-Reactive Protein (CRP) and White Blood Count (WBC) measured from the same blood sample. A total of 1113 simultaneous determinations from 269 different patients were studied, with an overall mean of four measurements, per patient. Of these 269 patients, the mean value for PCT was 2,48ng/mL, for CRP 121,47 mg/L and for WBC 11,78 x 103/µL. In 25% of cases, PCT either kept up with increase or decrease in CRP and WBC, but for 36% CRP rose earlier and to a greater extent than PCT. In no case PCT was the earliest biomarker.

On 70% of our PCT measurements overall values were low (<0.5ng/mL), with higher PCT in ICU patients. Indeed, studies have shown that in the majority of the population, PCT is very low (<0.02ng/mL) for healthy individuals and low in patients with pure viral infections. Early on the COVID-19 Pandemic, some authors showed that PCT was five times higher for severe disease.

Although, for 59% of our cases, CRP and WBC combined, were more sensitive and rose earlier than PCT, this doesn´t corroborate other studies where PCT was found to be a more accurate, sensitive and precise diagnostic and prognostic biomarker.

Further studies could improve the defition of severe disease to include information about infection source, outcome for the patient and co-morbidities, in order to understand if the differences found between our conclusions and other results, could be thus explained.

|P87

CHARACTERIZATION OF THE MOLECULAR PROFILE OF BEE VENOM ALLERGY

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Background: Hymenoptera venom allergy (HVA) is a potentially fatal allergic condition. It is a frequent cause of anaphylaxis in adults and children and is also responsible for decreased quality of life and significant anxiety about future stings. The best characterized hymenoptera venom is that of the bee (Apis mellifera). Several major allergens such as Api m 1, Api m 2, Api m 3, Api m 5 and Api m 10 when taken together show a diagnostic sensitivity of 95%: Api m 1 (phospholipase A2) is the most frequent molecular allergen in patients with Bee Venom (BV) allergy; Api m 3 (acid phosphatase) and Api m 10 (icarapin) are also considered specific to BV allergy; Api m 2 (hyaluronidase) shares 55% sequence identity with vespid hyaluronidase and Api m 5 (dipeptidyl peptidase 4) seems to be responsible for the cross-reactivity between bees and wasps. This study aims to characterize the sensitization profile by molecular components of patients with anaphylactic reactions to BV.

Methods: Retrospective study of patients with history of systemic reactions to bee sting, including skin testing and/or sIgE antibodies with whole extracts (Apis mellifera), between July 2016 and November 2020. Serum IgE to recombinant allergens (rApi m 1, rApi m 2, rApi m 3, rApi m 5, rApi m 10) by ImmunoCAP® (Thermo Fisher Scientific®, Uppsala, Sweden). Value ≥0.35 kUA /l was considered positive.

Results: Fourteen patients were included, 78.6% male, 21.4% female, mean age 48.5 years. Serum IgE to rApi m 1 was detected in 42.9% (6/14), rApi m 2 in 21.4% (3/14), rApi m 3 in 0% (0/14), rApi m 5 in 28.6 % (4/14), rApi m 10 in 35.7% (5/14) of patients. Seven patients (50.0%) were double positive and negative results for allergens were detected in 3 patients (21.4%).

Discussion: In vitro diagnostic of HVA allows an improved differentiation of the relevant sensitization, particularly in BV allergy, because it enables us to characterize individual sensitization profiles that may be of relevance for the treatment outcome of venom immunotherapy (VIT). Increasing the number of allergens available for molecular characterization of sensitization profiles improves diagnostic accuracy and combined with the use of cross-reactive carbohydrate determinant-free recombinant allergens allows differentiation between true sensitization and cross-reactivity. Venom immunotherapy (VIT) is the only long-term curative treatment available, and Api m 10 is considered a marker of failure in BV immunotherapy.