EVENTO ONLINE

13ª REUNIÃO CIENTÍFICA DA SPML

27 a 29
de Maio
de 2021

SPML
Sociedade Portuguesa
de Medicina Laboratorial

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PROGRAMA & RESUMOS

27.MAIO | 5ª FEIRA

16H00 – 17H00

SIMPÓSIO THE BINDINGSITE

CONTRIBUTO DOS BIOMARCADORES DE NOVA GERAÇÃO PARA A IMPLEMENTAÇÃO DA MEDICINA DE PRECISÃO NO CONTEXTO DO MIELOMA MÚLTIPLO

Moderador: Fátima Vale (ULS Guarda)

The current role of HLC in MM: what do the data tell us?
Rafael Ríos (Hospital Universitário Hospital Virgen de las Nieves)

Contributo do laboratório para um melhor seguimento da componente monoclonal
Carmen Quiñones-Torrelo (Hospital Clinic, Valencia)

17H00 – 18H00

SIMPÓSIO WERFEN

HEPARIN-INDUCED THROMBOCYTOPENIA: CLINICAL AND LABORATORY APPROACH

Moderador: Ana Cristina Oliveira (Clinical Specialist Werfen Iberia)
Palestrante: Rossella Marcucci (Florence -Careggi Hospital)

18H00 – 18H15 – PAUSA

18H15 – 19H15

SIMPÓSIO SPML

AVALIAÇÃO IMUNOLÓGICA EM TRANSPLANTAÇÃO RENAL

Moderador: André Weigert (CHLO)

18h15 – 18h30 **Métodos Inovadores de Monitorização Imunológica pós-transplante renal**
Cristina Jorge (CHLO)

18h30 – 18h45 **Marcadores virais utilizados na monitorização do estado imunológico dos doentes transplantados renais |** Sara Querido (CHLO)

18h45 – 19h00 **Anticorpos antiHLA na alocação de enxertos renais e na monitorização pós Transplantação renal |** Luís Ramalhete (IPST)

19h00 – 19h15 Discussão

19H15 – 20H15

SIMPÓSIO SPML

CIRURGIA OBESIDADE

Moderadores: Luísa Espinhaço (CHEDV), John Preto (CHUSJ)

19h15 – 19h40 **Tratamento Cirúrgico da Obesidade|** Mário Nora (CHEDV)

19h40 – 20h05 **Avaliação médica antes e após cirurgia para a obesidade|** Mariana Monteiro (ICBAS)

20h05 – 20h15 Discussão

28.MAIO | 6ª FEIRA

09H30 – 13H00

CURSO SPML
(MEDIANTE INSCRIÇÃO PRÉVIA)

CURSO DE ATUALIZAÇÃO EM PARASITOLOGIA
Teresa Baptista Fernandes (CHLO)

09H00 – 12H30

CURSO SPML
(MEDIANTE INSCRIÇÃO PRÉVIA)

WORKSHOP ON QUALITY ASSURANCE IN LABORATORY TESTING
Piet Meijer (ECAT Foundation)

16H00 – 17H00

SIMPÓSIO ABBOTT
VACINAÇÃO COVID 19
AVALIAÇÃO SEROLÓGICA – CHUC
Moderador: Helena Silva (Abbott Laboratórios)
Apresentador: Pedro Pereira (Abbott Laboratórios)
Palestrante: Lucília Araújo (CHUC)

17H00 – 18H00

SIMPÓSIO ROCHE
LIÇÕES DA PANDEMIA PARA PREPARAR O FUTURO
Moderador: Carlos Catalão (Roche)
Palestrantes: Tiago Guimarães (CHUSJ)
Carlos Sousa (UniLabs)
Carlos Cardoso (Lab. Joaquim Chaves Saúde)
João Faro Viana (CHLO)

18H00 – 18H15 **PAUSA**

18H15 – 19H15

10ª CONFERÊNCIA PROFESSOR ALBERTO AGUIAR
Moderador: João Tiago Guimarães (CHUSJ)

Updates on COVID-19 diagnostics?
Giuseppe Lippi (University Hospital of Verona)

19H15 – 20H15

SIMPÓSIO SPML
COVID-19
Moderadores: José Melo Cristino (CHULN), Helena Ramos (CHUP)

19h15 – 19h40 **Apresentação dos resultados da 2ª fase do Inquérito Serológico Nacional à COVID-19 (ISN-COVID19)** | Ana Paula Rodrigues (INSA)

19h40 – 20h05 **COVID-19 vaccines** | Hélder Mota-Filipe (FFUL)

20h05 – 20h15 Discussão

29.MAIO | SÁBADO

08H15 – 09H30

SESSÃO COMUNICAÇÕES ORAIS RÁPIDAS

Moderadores: Rui Farinha (CHUSJ), Jorge Nunes de Oliveira (Laboratório JNO)

|CR1 | Patrícia da Cunha Rodrigues
Infective Endocarditis Caused by Abiotrophia Defectiva - Case Report

|CR2 | Ana Torgal
Nonconformities in First Trimester Combined Screening Test Requests – Dealing with Unaccomplished Goals

|CR3 | Teresa Perico Pina
Lipaemia Without Lipaemia – Concealed Monoclonal Protein?

|CR4 | Sandra Coelho
Determination of Preanalytical Uncertainty for Serum Lipid Metabolism Analytes

|CR5 | Margarida Peixinho
The Importance of New Methodologies in the Diagnosis of Human Infections – First Reported Case of Kerstersia Gyiorum in a Terciary Hospital

|CR6 | José Sousa-Baptista
Fulminant Blastoid Variant of Mantle Cell Lymphoma with Leukemic Presentation – A Case Report

|CR7 | Paulo António Rodrigues Pereira
Sigma Metrics Established on the Number of Defects per Million Opportunities to Compute the Medical Laboratory Capability Index

|CR8 | Ana Spínola
Acquired Factor XI Deficiency: Clinically Relevant?

|CR9 | Paulo António Rodrigues Pereira
Internal Control of Binary Ordinal Quantities based on Loss of Clinical Sensitivity: Application to the Screening of Infectious Diseases

09H30 – 10H30

SIMPÓSIO QUILABAN

E DEPOIS DA INFEÇÃO… A IMUNIDADE!

NOVAS FERRAMENTAS PARA AVALIAÇÃO DA IMUNIDADE CELULAR DO SARS-COV-2

Moderador: Bruno Taveira (Quilaban)

Após infeção a imunidade: Importância dos estudos de imunidade no contexto do SARS-Cov-2.
Novas ferramentas no estudo imunidade celular: QuantiFERON SARS-CoV-2.
Diferentes aplicações do QuantiFERON SARS-CoV-2.
João Lacerda (QuantiFERON Team leader Iberia – Qiagen)

29.MAIO | SÁBADO

10H30 – 11H40

SESSÃO PRÉMIO POSTER SPML

Moderadores: Sandra Rebelo (CHUSJ), Maria Ana Pessanha (CHLO)

|P01 | Sara Sousa
Histiocytic Cells In Peripheral Blood Smear - An Alert For The Diagnosis Of Intravascular Large B-Cell Lymphoma

|P02 | Sandra Monteiro
WBC Scattergram, a Suggestion of Unstable Hemoglobins

|P03 |Ana Catarina B. Marques
Is Arterial Blood Gas Hemoglobin Trustable, Especially When its Value Drops Hard?

|P04 |Carolina Manco
Anaemia Obscured by Severe Hypertriglyceridemia

|P05 | Rita Oliveira
One Year of Covid-19 in Pediatric Age Group at Centro Hospitalar Universitário de São João

|P06 | Ana Faria
Result of the Portuguese Pilot EQA Program in Sars-Cov-2, PCR

|P07 | João Pinto
Rare Form of Acute Hepatitis Due to Herpes Simplex 2 Virus Infection after Kidney Transplantation

|P08 |Anunciação Ruivo
Robust and Age Dependent Immunologic Response of Healthcare Professionals Vaccinated with the Pfizer-Biontech Covid-19 Vaccine

|P09 | Tânia Cardoso
Urinary Free Cortisol Measurement: Comparison of Two Automated Immunoassays

|P10 | Dulce Alves Martins
Diagnostic Utility of the Immunocap Test For Anti-Pigeon Igg in Bird-Related Hypersensitivity Pneumonitis

11H40 – 12H00 **PAUSA**

12H00 – 13H20

SESSÃO PRÉMIO COMUNICAÇÃO ORAL SPML

Moderadores: João Faro Viana (CHLO), Cristina Marques (FFUL)

|CO1 | António Sarmento
Reference Values of Haematological Ratios in a Healthy Adult Population: the Epiteen Cohort Study.

|CO2 | Sofia Bastos Carvalho
New Formula for β-Thalassemia Screening

|CO3 | Manuel António de Azevedo Matos Garrido
The Prostate Health Index (PHI) Density in Prostate Cancer Detection: does it Outperform PHI or the Prostate-Specific Antigen (PSA) Density?

|CO4 | Mariana Marques

Importance of The Peripheral Smear in the Correct Evaluation of Basophilia – Alder–Reilly Inclusions in Maroteaux–Lemy Syndrome

|CO5 | Bruna Malheiro

Cold Agglutinins in The Context Of COVID-19

|CO6 | Vasco Mendes

Comparison of ADAMTS13 Activity Measurement by Elisa and a New Chemiluminescence Assay

|CO7 | Maria Luís Guerra

Value of Kappa Free Light Chain as a Biomarker In CSF Analysis for Multiple Sclerosis Diagnosis in three Centers from Portugal

|CO8 | Rita R. Lima

First-Trimester Combined Screening: one or two Step Approach?

13H20 – 13H30

ENCERRAMENTO E PRÉMIOS

COMISSÃO CIENTÍFICA

Adriana Pedrosa
Ana Paula Azevedo
Ana Paula Castro
Ana Paula Faria
Elsa Gonçalves
Eulália Costa
Fátima Vale
Fernando Rodrigues
Helena Brízido
João Mário Figueira
Manuela Ribeiro
Maria Jorge Arroz
Maria José Teles
Maria Luís Queirós
Rosário Luís

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NOTAS CURRICULARES & RESUMO APRESENTAÇÕES

| 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.

| Mário Nora

Since 01.09.2018 - Department Director, Centro Hospitalar de Entre o Douro e Vouga, Santa Maria da Feira (Portugal), Director of 1st Centro de Responsabilidade Integrada para o Tratamento Cirúrgico da Obesidade

Since 01.01.2009 - Department Director, Centro Hospitalar de Entre o Douro e Vouga, Santa Maria da Feira (Portugal), General Surgery Department Director

From 01.01.2001 until 31.12.2006 - Department Director, Hospital São Sebastião, Santa Maria da Feira (Portugal), General Surgery Department Director

Since 01.10.2004 - Department Director, Centro de Cirurgia Experimental Avançada, Founder and Technical Director

The author addresses the relationship between obesity, the digestive tract and the brain.

He presents a personal view how bariatric surgery alters this relationship and presents the results of the group he directs.

| 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.

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| 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.

COMUNICAÇÕES ORAIS
&
POSTERS

SESSÃO PRÉMIO COMUNICAÇÃO ORAL SPML

|CO1

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

António Sarmento, MD MSc[1]; Mariana Fragão-Marques, MD MSc[1]; Gustavo Mendes[2]; Maria José Teles, MD[1]; João Tiago Guimarães, MD PhD[3]

1Hospital de São João
2Faculdade de Medicina da Universidade do Porto
3Hospital de São João; ISPUP

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

Sofia Bastos Carvalho[1], Marta Fernandes[1]; Cacilda Magalhães[1]; Yuliana O. Eremina[2]

[1]Hospital Pedro Hispano, Unidade Local de Saúde de Matosinhos, E.P.E.;
[2]Hospital Pedro Hispano, Unidade Local de Saúde de Matosinhos, E.P.E.; EPIUnit, Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal

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?

Manuel Matos Garrido[1], Ruy Miguel Ribeiro[2]; Luís Campos Pinheiro[3]; Stefan Holdenrieder[4]; João Tiago Guimarães[5]

[1]Department of Clinical Pathology, Centro Hospitalar Universitário de Lisboa Central & Department of Laboratory Medicine, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal
[2]Biomathematics Laboratory, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal
[3]Department of Urology, Centro Hospitalar Universitário de Lisboa Central & Department of Urology, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisbon, Portugal
[4]Institute of Laboratory Medicine, Munich Biomarker Research Center, Deutsches Herzzentrum München, Technische Universität München, Munich, Germany
[5]Department of Clinical Pathology, Centro Hospitalar Universitário de São João; Department of Biomedicine, Faculdade de Medicina & EPIUnit, Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal

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)×√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.

SESSÃO COMUNICAÇÕES ORAIS RÁPIDAS

|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 preanalytical, 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.

SESSÃO PRÉMIO MELHOR POSTER SPML

|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 37°C. 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.

|P21

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.

|P22

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.

|P23

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.

|P24

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 lockdowns) are having a beneficial impact on the prevention of other respiratory diseases.

|P25

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.

|P26

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.

|P27

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.

|P29

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 corticotherapy, 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.

|P30

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.

|P31

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.

|P34

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., preeclampsia, 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 honour, 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 Immage ®. 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.

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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; Greatly supported by the scientific community.	Does not take into account the state of health of the patient and associated pathologies; 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.