| SESSÃO PRÉMIO MELHOR COMUNICAÇÃO ORAL

CO1

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

Lúcia Vieira[1], Rita Pombal[1], Sara Lopes[1], Joana Ribeiro[1], António Magalhães[1], Manuel Figueiredo[1]

[1]*Serviço de Imunohemoterapia, Centro Hospitalar Vila Nova de Gaia/Espinho*

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

Case Report: A G3P2A1 60-year-old woman with a uterine prolapse was proposed for a hysterectomy. A pre-operative type and screen was requested and a discrepancy was observed in the determination of ABO group. At room temperature, using Bio-Rad "DiaClon ABO/D" cards, no agglutination of the patient's red blood cells (RBC) occurred with anti-A and anti-B test sera. A very weak (<1+) agglutination was observed with anti-AB test serum. In the reverse typing, anti-B isoagglutinins were detected (4+), whereas anti-A1 isoagglutinins were not. Absence of anti-A2 isoagglutinins was also verified. At 4ºC, a very weak (<1+) reaction was observed with A_1 RBC and patient's serum. Both direct and indirect antiglobulin tests were negative.
A weak subgroup of A was suspected and the ABO genotype was determined, using a sequence-specific primer polymerase chain reaction (SSP-PCR). The genotype ABO:O1A2 was determined, predicting an A_2 phenotype. However, this phenotype typically presents a clear agglutination of patient's RBC in the presence of anti-A and anti-AB test sera, not consistent with the findings of this case.

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

CO2

REFRACTORINESS TO PLATELETS TRANSFUSION: IMMUNE OR NON-IMMUNE?

Rita Pombal[1], Lúcia Vieira[1], Joana Ribeiro[1], Helena Gomes[1], Manuel Figueiredo[1]

[1]*Centro Hospitalar Vila Nova de Gaia/Espinho*

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

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

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

CO3

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

Sofia Bastos Carvalho[1], Mafalda Felgueiras[1], Valquíria Alves[1]

[1]Serviço de Patologia Clínica, ULS Matosinhos - Hospital Pedro Hispano

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

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

Material e Methods: A prospective study was performed from 1st November to 31st December of 2020. A total of 269 urine samples were evaluated. The samples were collected in sterile urine cups. At the delivery time of the urines at the health care centers, a sample was taken from each urine into a new sterile cup with boric acid (BA) and then sent to the laboratory. On all the samples, a standard quantitative urine culture was performed. Cultures with growth $\geq 10^5$ CFU/mL (colony forming unit per millilitre) were considered positive. Furthermore, all the urine samples were used for the UF-5000 analysis. The diagnostic accuracy of UF-5000 was examined for a range of cut-offs for the bacterial (BACT) and leukocytes (WBC) count. The best cut-off point was obtained from the ROC curve analysis to maximize the negative predictive value (NPV) and the best screening rate. Statistical analysis was performed with the software *GraphPad Prism* (version 5.0). The level of significance was set at $p<0.05$.

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

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

CO4

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

João Pedro Barreto[1], Ana Marta Pires[1], Patrícia Valério[2], Teresa Chuva[1], Carla Azevedo[1], Hugo Ferreira[1], Ana Paiva[1], Gabriela Martins[1], José Maximino Costa[1]

[1]IPO Porto, [2]Centro Hospitalar de Setúbal

Introduction: Severe kidney injury (mainly due to light chain deposition, cast formation and tubular obstruction) is observed in up to 20% of multiple myeloma (MM) patients, of which up to 5% need dialysis. High cut off dialysis may reduce the levels of circulating monoclonal free light chains (FLC).
A sustained reduction in FLC (through chemotherapy and hemodialysis) has been associated with the recovery of kidney function and better survival.

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

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

Results/Discussion: In all patients, the dialytic technique effectively removed serum FLC (average rate of removal per patient per session: 35%, 51% and 55% for kappa; 20%, 22% and 28% for lambda). The lower rates for lambda may be due to different conformation (dimeric/polymeric) and higher molecular weight. Higher percentages of removal may not be attainable due to *in vivo* serum replenishment from extravascular compartment.
Measurements in the ultrafiltrate (pre- and post-adsorption filter) showed an effectiveness of nearly 99% in the beginning of dialysis for both kappa and lambda FLC. Approaching the end of the session, a filtration of 93% was obtained for kappa FLC, while a drop to 52% was observed for lambda FLC. Filter saturation could account for this variation, in particular for lambda FLC. Although ultrafiltrate is not a validated matrix for Freelite®, this data seems plausible.

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

CO5

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

Patrícia da Cunha Rodrigues[1], Fernando Branca[1], Aurélio Mesquita[1]

[1]*Hospital de Braga*

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

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

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

Results: A total of 188 samples from 153 patients were included in the study.
There were 98 samples (52.2%) with a positive result on the BF-PP.
On the negative BF-PP group, 56 samples (62.2%) had a negative cultural test, 29 (32.2%) and 5 (5.6%) had, respectively, cultural isolation of agents not included and included in the BF-PP.
Of the samples with a positive result in BF-PP, a single agent was identified in 52 samples, two agents in 23, three agents in 17 and four agents in 4 samples.
There were 28 samples (28.6%) with entirely concordant positive result. Microbiological culture was negative in 21 samples (21.4%) with positive BF-PP. Fungi or bacteria not included in the panel were isolated in 19 samples (19.4%). There was partial agreement (positive culture for some agents in BF-PP) in 28 samples (28.6%). In 2 samples (2.0%) an agent was isolated in culture, not identified by BF-PP.
The positive percentage agreement between the BF-PP and the culture was 91.5% (82.5 - 96.8) and the negative percentage agreement was 96.5% (95.7 – 97.1).

Conclusions: BF-PP is a useful tool for quick diagnostic of lower respiratory infections in ICU patients. However, it does not replace culture because of pneumonia agents that are not included in the BF-PP. In addition, a comprehensive antibiotic susceptibility test is only possible through culture methods.
BF-PP has high agreement with culture. The discrepant results can be explained by the greater sensitivity of molecular methods, the antibiotic therapy prior to specimen collection and the fastidious nature of certain bacterial agents.

CO6

ATYPICAL HEMOLYTIC UREMIC SYNDROME: A CASE REPORT

Ana Aguiar[1], Mariana Carmona[2], Filipa Rodrigues dos Santos[3], Joao Pedro Barreto[4], Ivete Lima[5], Carolina Carneiro[6], Lídia Branco[5], Julia Vasconcelos[5], Graça Franchini[5], Josefina Santos[7], Esmeralda Neves[5]

[1]*Serviço de Patologia Clínica Centro Hospitalar Entre Douro e Vouga*
[2]*Serviço de Patologia Clínica Unidade Local Saúde Nordeste*
[3]*Serviço de Imunoalergologia Centro Hospitalar Universitário do Porto*
[4]*Serviço de Patologia Clinica Instituto Portugues do Porto*
[5]*Serviço Imunologia Centro Hospitalar Universitário do Porto*
[6]*Serviço Imunologia, Centro Hospitalar Universitário do Porto*
[7]*Serviço Nefrologia Centro Hospitalar Universitário do Porto*

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

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

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

CO7

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

Paulo Pereira[1]

[1]*Instituto Português do Sangue e da Transplantação, I.P.*

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

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

This guide reviews and introduces important principles in qualitative performance assessment. The evaluation is mainly based on Bayesian probability, through clinical sensitivity and specificity, from the perspective of the medical laboratory. The guide also reviews the agreement of binary results for cases where the condition is unknown, such as unknown diagnosis. The predictive values are reviewed if we

consider the physician's view (clinical decision). An important concept introduced in this guide is the uncertainty of proportions, such as clinical sensitivity and specificity uncertainty. The uncertainty interval is estimated based on the 95% confidence interval principle. We can interpret it as the measurement of the chance of a given probability happening.
The guide is designed to apply to various areas of chemistry, including qualitative testing in the medical laboratory. The example of the performance assessment of an RT-PCR SARS-Cov-2 RNA test is a mere application that appears in the guide. It can be replicated for all qualitative tests with binary nominal quantities, such as true/false, positive/negative.

CO8

CO-INFECTION WITH SARS-COV-2 AND INFLUENZA A VIRUS IN CHILDREN Tipo: Comunicação Livre

Rui Figueiredo[1], Afonso Santos[1], Helder Alves[1], Luís Morais[1], Paula Gama[1], Carlos Cortes[1]

[1]*Centro Hospitalar do Médio Tejo*

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

Objective: To characterize Coviflu cases in children.

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

Results: From 492 patients under 18 y/o, 25 (5%) presented Influenza A virus type H3 (AH3), and 4 (0.8%) had also SARS-CoV-2 co-infection. Patients with Flu A virus, were mostly men (n=16; 64%). Coviflu infection were observed in 3 men (A, C, D), aged with 1, 6 and 9 y/o, and 1 female (B), aged 15 y/o (Table 1). Patient A revealing slight fever (37.7 ºC), cough, and rhinorrhea were also co-infected with adenovirus, and coronavirus 229E. Patient B showing cough, fever (39.2ºC), vomiting, and muscle pain; and patient C with fever (38.7ºC) and headache had only identified with Coviflu. Patient D with fever were co-infected with coronavirus 229E, and Rhino/Enterovirus too. No internment was necessary. All had SARS-CoV-2 Ómicron variant, but patient A and B the BA.2 sub-lineage, and patients C and D BA.1 sub-lineage.

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

CO9

IMMUNE RESPONSE AFTER FULL SARS-COV-2 VACCINATION

Tiago Ramalho[1], Rúben Costa[1], Eduarda Lopes[1], Bianca Braz[1], Viviana Martins[1], Márcia Gonçalves[1], Sara Guedes[1], Carina Ribeiro[1], Ana Moreira[1], Susana Gomes[1], Mafalda Felgueiras[1], Rosário Alves[1], Carlos Soares[1]

[1]*Serviço de Patologia Clínica, ULS Matosinhos - Hospital Pedro Hispano*

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

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

Materials and Methods: An evaluation of acquired immunity by determination of anti-spike glycoprotein immunoglobulin levels, via Chemiluminescent Microparticle Immunoassay (CMIA) (Abbott Architect i2000SR), in serum samples collected from healthcare workers was conducted. Antibody levels were determined at 3 timepoints: Pfizer® vaccine (PF): T0 – day of 1st dose administration; T1 – 21 days after the 2nd dose; T2 – 6 months after T0. AstraZeneca® vaccine (ATZ): T0 – day of 1st dose administration; T1 – 1 month after the 2nd dose; T2 – 6 months after T0. An IgG result >50 AU/mL was considered positive for immunity.
380 participants were included: 213 were vaccinated with PF, 167 were vaccinated with ATZ. Exclusion criteria: positive immunity at T0 (n=21), negative immunity at T1 (n=2) or T2 (n=4), and participants that received different brands for each dose (n=15). At each timepoint, the median and respective quartiles [P25; P75] were calculated using GraphPad Prism (version 5.0). The significance level was set at $p<0.05$.

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

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

CO10

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

Margarida Peixinho[1], Anália Carmo[1], Fernando Rodrigues[1], Eulália Costa[1], Ana Cristina Marques[1], Ana Isabel Rodrigues[1]

[1]*CHUC*

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

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

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

Results: 71% of patients were male and 29% female, with a mean age of 52±12.9 years. 58% of patients performed a search of HFE gene: 11% were homozygous for H63D, 11% were heterozygous for C282Y, and 22% were heterozygous for H63D. Only in three patients was performed the search for the mutation in the UROD gene and only one was heterozygous for c942G>A. At the diagnosis, the laboratory results were: ferritin 566 ± 371ng/ml, uroporphyrins 1163 ± 1138ug/24h, coproporphyrins 770 ±362ug/24h and porphyrins 1739 ±1288ug/24h. In the exacerbations the most frequent precipitating factors were: alcoholism (39%), Hepatitis C virus (HCV) (16%), estrogen use (3%), HIV associated with HCV and alcohol use (6%). Patients with HCV are those with most elevated mean values of uroporphyrins (3655.3±909ug/24h).
Regarding the treatment, 29% of patients were not submitted to any type of therapy, 25.8% underwent phlebotomies and only one patient was treated with plaquinol. 70% of patients presented a significant reduction of ferritin upon treatment (-68.9±19.1%).

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

CO11

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

Tiago Ramalho[1], Mafalda Felgueiras[1], Carolina Domingues[1]

[1]*Serviço de Patologia Clínica, ULS Matosinhos - Hospital Pedro Hispano*

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

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

Materials and Methods: A retrospective study was done based on simultaneous Ca and Alb measurements of 9165 patients. Both measurements were performed using the Abbott Architect ci8200 by Arsenazo III (Ca) and bromocresol green (Alb) methods.
Exclusion criteria: age < 18 years, creatinine > 2.26 mg/dL, Alb < 2.0 g/dL or > 5.0 g/dL, total Ca > 12 mg/dL, parathyroid hormone, alkaline phosphatase and/or alanine transaminase above the reference value.
The population studied was randomly divided into two cohorts: derivation cohort with 75% of the samples (nd=6874); validation cohort with 25% (nv=2291). Simple linear regression associating Ca and Alb was constructed from the data in the derivation group, which was subsequently validated in the validation group. Our equation performance was later compared with the most frequently used equation (Payne's formula) A*djusted[Ca](mg/dL) = Total[Ca](mg/dL) + 0,8(4,0 - [Alb](g/dL)*, in 1354 subjects with hypoalbuminemia (Alb < 3,4 g/dL).

Results: From the analysis of our data, we obtained the equation *Adjusted [Ca](mg/dL) = Total [Ca](mg/dL) + 0,803 (3,979 - [Alb](g/dL))*, which showed good internal validity (adjusted r^2 shrinkage = -0,022).
When comparing the Ca status values obtained in individuals with hypoalbuminemia (n=1354) using the two equations, we found that only 14 were not in agreement (weighted kappa = 0.99), revealing very good agreement.

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

CO12

CASE REPORT - IS IT ONLY MALARIA?

Luís Lara Albuquerque[1], Raque Afonso Conde[1], João Oliveira Neves[1], Aurélio Mesquita[1]

[1]*Hospital de Braga*

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

Clinical case: A Cape Verde 13-year-old female, resident in Nigeria for 1 year, has been in Portugal for 15 days and went to the emergency department in January 2022 presenting fever with 3 days of evolution, associated with generalized headache, vomiting and back pain, with gait limitation. In the last 12 months, she had 2 episodes of malaria, the last one in October, with no other relevant history.
The blood count showed normocytic normochromic anemia, leukopenia with lymphopenia and thrombocytopenia. She presented with liver function aggravation and the increase of D-Dimers, CRP, PCT and VS. Blood culture and urinary sediment were negative. She showed positive Paul-Bunnel, positive blood smear for *Plasmodium* (1.5% parasitemia) and positive *P. falciparum* antigen. Treatment with artemether/lumefantrine was initiated, considering Nigeria as an endemic area of chloroquine resistance.
During hospitalization, analytical aggravation of anemia, leukopenia, thrombocytopenia and hepatic markers were observed, having been transfused with hemoderivatives and transferred to CHUSJ due to hemodynamic instability.
Immunological study was positive for VCA EBV IgG and EBNA, IgM *Mycoplasma*, anti-*Borrelia B.Burgdorferi* IgG and IgM, and IgG antibodies to Dengue virus. Confirmation of anti-*Borrelia* by *Western-Blot* was IgG and IgM positive.
To confirm these findings, it was performed a study of *Plasmodium*, *Borrelia* and EBV by RT-PCR, which was positive only for *Plasmodium* spp.

Discussion: A cross-reaction happens when an antibody binds to an antigen for which it was not specifically produced, since it has correlated structures. Thus, it is possible for antibodies to react to antigens without prior exposure.
The presence of vectors for *Borrelia* in Nigeria and Cape Verde is not described in the literature. The most frequent cause for this type of findings is intravenous gammaglobulin therapy, which was not observed.
A diagnosis comprehends the laboratory findings and the clinical diagnostic methods, such as clinical markers, physical examination and imaging techniques. Most important is a consideration of the final clinical picture to decide if the laboratory results fit the clinical and endemic context of the patient.

| SESSÃO COMUNICAÇÕES ORAIS RÁPIDAS

CR1

COVID-19 SEVERITY ACCORDINGLY SARS-COV-2 VARIANTS

Afonso Santos[1], Rui Figueiredo[1], Luís Morais[1], Paula Gama[1], Emilia Krasteva[1], Carlos Cortes[1]

[1]*Centro Hospitalar Médio Tejo*

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

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

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

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

CR2

CANCER DIAGNOSIS – WHEN THE COAGULATION STUDY IS THE KEY

Lúcia Vieira[1], Rita Pombal[1], Joana Ribeiro[1], António Magalhães[1], Manuel Figueiredo[1]

[1]*Serviço de Imunohemoterapia, Centro Hospitalar Vila Nova de Gaia/Espinho*

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

Case report: A 43-year-old woman underwent a surgical exodontia. In the following 2 weeks, she went back to the hospital for 4 times due to persistent oral bleeding. The transfusion of 2 units of packed red blood cells was needed. The patient also reported menorrhagia in that month. Three weeks after the procedure, she was admitted to the emergency department with cough, fever and severe asthenia, presented over the past 6 days. The physical examination revealed a peripheral oxygen saturation of 60%. An arterial blood gas test was performed, revealing a severe respiratory failure (pO_2 33,5 mmHg), with hypocapnia (pCO_2 24,9 mmHg). These findings, associated with atypical abnormalities of the chest X-ray, led to the diagnosis of pneumonia and antibiotic initiation. A coagulation study was also requested: prothrombin time 23 seconds (normal: 11,5-14,5), activated partial thromboplastin time 43,9 seconds (normal: 24,0-34,0). In front of this study and after investigation in the electronical process, the clinician of the Immunohemotherapy department decided to add fibrinogen assay to the requested study, considering the bleeding history. A fibrinogen level of 48 mg/dL (normal: 200-400) was determined. D-dimers >20 µg/mL (positive if >0.5) were also assessed, with a platelet count of 155000/mm^3. A massive pulmonary embolism or a disseminated intravascular coagulation (DIC) were considered as the main diagnosis hypotheses. Thus, a thoracic computed tomography was performed, revealing a probable pulmonary neoplasia, with diffuse metastases (liver, bone and ganglia). A paraneoplastic DIC was, therefore, assumed as the most probable explanation to the coagulation study findings.

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

CR3

MICROSCOPIC HEMATURIA IN ADULTS

Vítor Manuel de Azevedo Soares[1], Henrique Borges[2], Teresa Jerónimo[2], Vítor Teixeira[3], Delminda Simões[1], Ana Paula Soares[2], Nélio Santos[1]

[1]Serviço de Patologia Clínica, Centro Hospitalar Universitário do Algarve, E.P.E. - Unidade de Faro
[2]Serviço de Nefrologia, Centro Hospitalar Universitário do Algarve, E.P.E. - Unidade de Faro
[3]Serviço de Reumatologia, Centro Hospitalar Universitário do Algarve, E.P.E. - Unidade de Faro

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

Clinical Case Report: We present the case of a 68-year-old woman with asymptomatic isolated microscopic hematuria (RBCs 5-9/HPF) found in a routine urine sample analysis with a decline in GFR (40mL/min/1,73m^2) and a previous history of hypothyroidism, hypertension and dyslipidemia. Further tests showed an urinary sediment with RBCs 20/HPF, dysmorphic RBCs (10%), proteinuria (278 mg/24 hours),

positive antineutrophil cytoplasmic antibodies (ANCA)–myeloperoxidase (MPO), and antibodies against thyroid peroxidase. She was admitted in the Nephrology department of the hospital to perform a renal biopsy, which revealed a pauci-immune necrotizing glomerulonephritis with cellular crescents. She started treatment with methylprednisolone pulses for three days, oral prednisolone, 1mg/kg/day and cyclophosphamide. Due to the fact of hyponatremia and suspicion of cyclophosphamide cardiac toxicity, a switch was made to rituximab. She currently has a stable GFR of 37mL/min/1,73m^2, without proteinuria and RBCs 7/HPF with 4% dysmorphic RBCs.

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

CR4

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

Mariana Benita Afonso[1], Beatriz Araújo[1], Susana Martins[1], Gorett Carvalho[1], Ana Paula Barbosa[1], Aurélio Mesquita[1], António Marques[1]

[1]*Hospital de Braga*

Clinical report: 25-year-old female, black race, living in Portugal for six years. Personal history of chronic normocytic anaemia of unknown aetiology since 2020, requiring blood transfusions. Admitted to the emergency department in 2022 due to asthenia, anorexia, nausea/vomiting, which she stated having been present for five days. There were no complaints of fever, blood loss, neither other neurological/respiratory/gastrointestinal symptoms.
On physical examination, she presented with jaundice and pallor. Anaemia (haemoglobin 8.2g/dl, mean blood volume 98.8 fL, reticulocytes 2.2%) was documented on the hemogram. The peripheral blood smear showed marked anisopoikilocytosis with dacrocytes and schizocytes. Additionally, the blood study revealed increased lactate dehydrogenase (5097 U/L), bilirubin (direct 1.44 mg/dl /indirect 0.53 mg/dl) and decreased haptoglobin (< 1%), suggesting haemolytic anaemia.
Given her clinical condition and the laboratory findings, she was hospitalized for aetiological study, which revealed low vitamin B12 (124 pg/mL) and negative direct Coombs test, hemoglobin H test and osmotic fragility test. The red blood cells pathology study using the methodology HPLC (high-performance liquid chromatography) did not show any variant or relevant alteration. Other infectious and neoplastic causes were excluded.

Discussion: As the patient presented with vitamin B12 deficiency without any dietary restrictions, atrophic gastritis was investigated and confirmed by endoscopic gastric biopsy [positive anti-parietal cell antibodies (title 1/40) and low intrinsic factor (31 RU/ml)].
The pernicious anaemia hypothesis was supported by the analytical and clinical response to the administration of intramuscular vitamin B12 [improvement in reticulocytes (10.5%) and hemoglobin (9.9 g/dl) values and haptoglobin normalization (52 mg/dl)].
The presence of laboratory criteria for hemolysis, justified by ineffective hematopoiesis, guided the study towards screening for a hemolytic cause which, in the case of a young woman, included congenital or acquired pathology of the red blood cell.
Although there were no neurological symptoms or typical hematological changes, B12 deficiency anemia was confirmed, being relevant to the exposure of these clinical cases that can be associated with high morbidity.

CR5

BLEEDING DIATHESIS AS THE INITIAL PRESENTATION OF ACUTE PROMYELOCYTIC LEUKEMIA

Hugo Alves[1], Ana Meireles[2], Lino Azevedo[1], Bruno Fernandes[2], Susana Fernandes[2], Cecilia Correia[2], Teresa Sousa[2], Carlos Mendes[2], Ana Paula Cruz[1], Gabriela Martins[2]

[1]*CHVNG*
[2]*IPOPORTO*

A 59-year-old male with a medical history of hypertension, dyslipidemia, and chronic obstructive pulmonary disease, in the context of alpha 1 antitrypsin deficiency, was admitted at the hospital due to new onset of hemorrhagic oral blisters and spontaneous hematomas dispersed throughout the body.
A complete blood count was performed and showed severe thrombocytopenia (27.000), and mild leukopenia (1,0 $x10^9$/L). The comprehensive metabolic panel was innocent. He had no constitutional symptoms, and C-reactive protein was negative. Abdominal and renal ultrasounds showed no relevant changes and no other significant physical examination findings were observed. Viral studies were negative for Hepatitis B, C, HIV, and SARS-CoV-2. Urinalysis showed hematuria. The examination of the peripheral blood smear revealed 2 % blast cells. Coagulation studies showed normal activated partial thromboplastin time, elevated prothrombin time (15,5 seconds), and an international normalized ratio of 1,32. D-dimers were > 20 ug/mL, and fibrinogen was decreased (144 mg/dL).
Acute leukemia was suspected, and the patient was admitted to start all-trans retinoic acid treatment. Bone marrow aspiration showed massive infiltration of promyelocytes/blasts with abundant Auer rods. Further immunophenotypic screening showed the presence of 84% of myeloid blasts that had a heterogeneous expression of CD13 and were CD 33+, CD 34-, CD 38+, CD 64+, MPO+. This immunophenotype was compatible with acute promyelocytic leukemia (APL). Fluorescence *in situ* hybridization analysis further revealed the presence of a PML-RARA fusion and the karyotype showed the reciprocal 15;17 translocation which is characteristic of the APL and confirmed the diagnosis.
Acute promyelocytic leukemia represents a medical emergency with a high rate of early mortality. It is necessary to start ATRA treatment without delay, as soon as the diagnosis is suspected, to decrease the risk of complications associated with APL coagulopathy. It is paramount for the clinical pathologist reviewing CBCs results to perform a blood smear evaluation and thoroughly evaluate the presence/absence of immature granulocytes and rule out APL, especially, when bi/pancytopenia presents with bleeding diathesis.

CR6

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

João Pedro Porto[1], Carolina Salteiro[1], Margarida Guimarães[1], Carlos Flores[1]

[1]*Centro Hospitalar e Universitário de Lisboa Central*

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

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

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

CR7

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

Teresa Almeida[1], João Tiago Guimarães[2], Sandra Rebelo[3]

[1]Serviço de Patologia Clínica, Centro Hospitalar Universitário de São João
[2]Serviço de Patologia Clínica, Centro Hospitalar e Universitário de São João; Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto; EPIUnit – Instituto de Saúde Pública, Universidade do Porto, Portugal
[3]Serviço de Patologia Clínica, Centro Hospitalar e Universitário de São João; Departamento de Biomedicina, Faculdade de Medicina da Universidade do Porto; Instituto de Investigação e Inovação em Saúde, i3S, Porto, Portugal

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

We retrospectively reviewed all laboratory results of viruses detected in respiratory specimens collected from children (0-18 years old) from 2018 to 2021, in a tertiary care Hospital in Portugal.
From 2018 to 2020, the results show a seasonal variation in viral infections, with peaks in the winter, between November and March – maximum number of cases 110 in January 2018, 101 in January and February 2019, 77 in January 2020. In the winter of 2020/21, the first winter after the pandemic, when preventive measures against COVID-19 were harsher, we observed an interruption of that pattern, with abnormally low numbers of infections (maximum 9 cases in January 2021. In contrast, during the summer of 2021, there was an unusual increase, coinciding with the relief of the restrictions when control of the infection by SARS-CoV-2 was better - 63 cases in July/August 2021 *vs* 1, 0 and 5 cases in 2018, 2019 and 2020, respectively.

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

CR8

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

José Marta[1], Gouveia Pereira[1], Tânia Lapão[1], Sofia Guitana[1], Pedrina Sousa[1], Vitória Matos[1], Carlos Flores[1]

[1]*Centro Hospitalar Universitário de Lisboa Central*

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

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

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

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

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

CR9

EXTERNAL QUALITY ASSESSMENT: TRENDS AND DEVELOPMENTS

Paulo Pereira[1]

[1]*Instituto Português do Sangue e da Transplantação, I.P.*

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

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

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

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

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

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

| SESSÃO PRÉMIO MELHOR POSTER

P01

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

Fátima Lamas[1], Anunciação Ruivo[1], Lara Santos[1], Marina Rocha[1], Teresa Campos[1], Virgínia Caetano[1], Filipa Silva[2]

[1]Immuno-hemotherapy Department, Centro Hospitalar do Tâmega e Sousa (CHTS), EPE, Penafiel, Portugal,
[2]Occupational Medicine Department, Centro Hospitalar do Tâmega e Sousa (CHTS), EPE, Penafiel, Portugal

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

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

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

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

P02

VARIATIONS IN HAIRY CELL LEUKEMIA-VARIANT: TWO CASE REPORTS

Bruno Filipe Oliveira Esteves[1], Patricia Amantegui[1]

[1]*Centro Hospitalar Universitário Cova da Beira*

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

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

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

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

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

In both cases, immunophenotypic analysis was a key tool for the diagnosis. The first case is particularly interesting because it shows an unusual immunophenotypic profile in which the hairy cells didn’t express neither lambda nor kappa light chains.
In conclusion, this work shows two unusual presentations of HCL-V that should be taken into account when considering this diagnosis.

P03

PRIMARY EFFUSION LYMPHOMA IN AN AIDS PATIENT: A CASE REPORT

Ricardo Verde[1], Ana Machado[1], Ahmed Gany[1], Jesuína Duarte[1], Ana Carvalho[1], Carla Antunes[1], Matilde Gonçalves[1]

[1]*Centro Hospitalar de Setúbal - Hospital de São Bernardo*

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

We report a case of a 47-year-old, man, with a medical history of mixed HIV ½ infection and with AIDS criteria (CDC C3), diagnosed in 2010, and with Kaposi's sarcoma in 2011, presenting to the emergency department (ED) with weight loss, anorexia, and progressive increase of the abdominal distension and constipation, over the last 3 weeks. The ultrasound confirmed the presence of ascites. He was admitted to the hospital for further study. During the ED stay, it is noteworthy to report the analysis of ascitic fluid, with 21600/µL leukocytes and "observation of 60% of large cells, lymphoid-like, prominent deeply basophilic cytoplasm, and several vacuoles, suggestive of high-grade non-Hodgkin's lymphoma". During hospital stay, it is noteworthy the ascitic fluid's immunophenotype: CD45+, CD19-, CD20-. It was initially excluded some types of B-cell Non-Hodgkin lymphoma, like Burkitt lymphoma and diffuse large B-cell lymphoma. Further study was done, and it was performed diagnostic laparoscopy. The ascitic fluid was sent to Clinical Pathology and Anatomical Pathology (AP). The AP report was: "The cells are large, with irregular hyperchromatic nucleus, some with nucleoli. The cells are CD45+; CD20-; CD79A; CD3-; AE1;AE3-; CD5-; HHV8 strongly +" These characteristics are compatible with Primary Effusion Lymphoma. The patient was referred to a hematologist-oncologist and is under therapeutic protocol.
Primary effusion lymphoma is a rare, aggressive disease with a poor prognosis. The role of the clinical pathologist is fundamental because they actively participate in the diagnosis of this disease. Even though the diagnosis was not made within the field of Clinical Pathology, the pathologist had a contributing role in the differential diagnosis.

P05

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

André Pinto[1], Ana Fontes[1], José Carvalho[1], Eliana Costa[1]

[1]*Centro Hospitalar de Trás-os-Montes e Alto Douro*

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

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

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

A total of urine samples from 7964 patients (hospitalized and outdoor) were the object of this study. In 5710 samples (71,7%) microorganisms (bacteria and yeast like cells) were not identified by FFC and the microbiology results were reported like negative (78,6%) or with no clinical relevance (21,4%) because the culture presented 3 or more different microorganisms. 2254 samples were reported by FFC with the presence of microorganisms. Among these, 956 with the existence of Gram Negative Bacilli (GNB) by cytometry and 897 had a microbiological exam with >10^5 CFU (Colony Forming Units) of BGN.

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

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P06

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

Edna Pereira[1], Anabela Santos Silva[1], Helena Correia[1], Susana Silva[1], Ana Faria[1]

[1]*Instituto Nacional de Saúde Dr. Ricardo Jorge*

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

Methodology: During 2007-2015 it was sent 89 positive and 72 negative slides of mycobacteriology microscopy and in 2016-2020 41 positive and 34 negative. The results were compared with the expected results and re-evaluated by the organizing Laboratory in case of discrepancy. This study included national public and private clinical laboratories as well as laboratories from Portuguese speaking countries (CPLP). The COVID-19 impact was made using the chi-squared test considering a significance level of 5%.

Results: In the first period analysed (2007-2015) there were 3,76% incorrect results corresponding to: quantification error (0,55%); false negative (2,27%) and false positive (0,94%) in 4817 results (89 positive [(8)1-9/100 fields; (38) 1+;(32) 2+;(11) 3+] and 72 negative).
During 2016-2020 there were 6,36% of incorrect results: quantification error (2,49%); false negative (2,49%) and false positive (1,38) in 1525 results (41 positive [(20) 1+; (16) 2+; (5) 3+] and 34 negative).
The samples analysed in pre-pandemic period (2017-2019), 24 positive [(11) 1+;(11) 2+;(2) 3+] and 21 negative, presented 94,54% correct results and 5,46% incorrect results – quantification error (1,98%); false negative (2,36%) and false positive (1,13%).
The samples analysed in the pandemic period (March 2020 - April 2021), 17 positive [(9) 1+;(5) 2+;(3) 3+] and 13 negative, presented 91,63% of correct results and 8,37% incorrect results – quantification error (3,65%); false negative (2,79%) and false positive (1,93%).
Significant differences between pre-pandemic and pandemic period was observed (p=0.03)

Conclusion: We observed a decrease in performance through the studied period, accentuated during the pandemic period, mainly with the increase of quantification error and false positive.
Comparing the performance from 2007-2015 with 2016-2020 we observe a decrease of performance during the recent years, also with an increase in quantification errors.
The decrease of performance observed in the last 4 years (2016-2020) reinforces the importance of participating in external quality assessment schemes and practical education.

P07

STREPTOCOCCUS PNEUMONIAE SEPSIS IN CHILDREN AFTER INTRODUCTION OF PNEUMOCOCCAL CONJUGATE VACCINES

Luís Morais[1], Rui Figueiredo[1], Helder Alves[1], Jóni Mota[1], Ana Nascimento[1], Paula Gama[1], Carlos Cortes[1]

[1]*Centro Hospitalar do Médio Tejo*

Key-words: Sepsis, *Streptococcus pneumoniae*, Children

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

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

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

Discussion and Conclusions: It was identified serotype 19F in one child. In this case *S. pneumoniae* is levofloxacin susceptible, increased exposure, penicillin and erythromycin resistant.
Another identified serotype was serotype 8. Serotype 8 isolate belonging to the 8-ST53 clone determinated by MLST. In this case *S. pneumoniae* is levofloxacine, peniciline and erytromicime susceptible.
The serotype is a key determinant of IPD potential and prevalence, however, analysis by MLST typing, contribute to the genetic characterization and understanding of their spread.
Currently, in countries of Western Europe in which the PCV13 vaccine is used, serotype 8 is one of the most frequently recovered, because it's not part of serotype polysaccharides (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) of the vaccine. These children fulfilled the Portuguese NVP 2020.
The incidence of pneumococcal sepsis in children remained high after the introduction of the pneumococcal conjugate vaccine (PCV)-13.
In conclusion, the first child appears to be a vaccine failure and the second does not, however, in both cases it caused invasive disease.

P08

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

Luis Miguel Lopes Martinho[1], Ana Cristina Salgado Silva[1], Mara Rodrigues[1], José António Peixoto Carvalho[1], Eliana Costa[1]

[1]*Centro Hospitalar de Trás-os-Montes e Alto Douro*

Background: Since the late 1990s, serum levels of Interleukin-6 (IL-6) have been used as a diagnostic for several inflammatory diseases [1]. IL-6 is a pleiotropic proinflammatory cytokine produced by a variety of cells including lymphocytes, monocytes and fibroblastos [2]. Its serum values rise rapidly in response to various pathological events, and are usually associated with prognoses of a severe course [(3-5)]. Recently, the

COVID-19 pandemic has elucidated another very important role played by this cytokine in the follow-up of patients, revealing that the dosage of IL-6 is able to guide the clinician regarding the severity of the disease and the eventual need for transfer to the Intensive Care Unit (ICU) [6]. SARS-CoV-2 infection induces a dose-dependent production of IL-6 from bronchial epithelial cells, causing systemic inflammation, leading to hypoxemic respiratory failure, which may be associated with the increased release of this particular cytokine. This biological phenomenon is called "cytokine storm"[7].

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

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

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

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P09

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

Lídia Carvalho[1], Luísa Lacerda[1], Manuela Ribeiro[1]

[1]*Centro Hospitalar Universitário de São João*

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

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

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

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

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

P10

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

Luís Morais[1], Rui Figueiredo[1], Hélder Alves[1], Jóni Mota[1], Ana Nascimento[1], Rita Coelho[1], Rosa Soares[1], Irina Bernardo[1], Márcia Almeida[1], Afonso Santos[1], Paula Gama[1], Carlos Cortes[1]

[1]*Centro Hospitalar do Médio Tejo*

Introduction: The lack of immunity to SARS-CoV-2 has led to rapid evolution since its discovery in December 2019. Various researchers hypothesized that live-attenuated vaccines as rubella, measles, and Bacillus Calmette-Guérin (BCG) can result in cross protection against severe COVID-19. Measles vaccination is done by live-attenuated, negative-stranded RNA virus. Measles vaccine is a lifetime vaccine. Pfizer/BioNTech vaccine (BNT162b2) is a lipid nanoparticle-formulated, nucleoside-modified RNA vaccine that encodes a perfusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein.

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

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

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

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

| POSTERS EM EXIBIÇÃO

P11

MIXED LYMPHOID/MYELOID LEUKEMIA IN PEDIATRICS - CLINICAL CASE

Ana Luísa Araújo[1], Pedro Vale[1], Ezequiel Moreira[1], Helena Ferreira Silva[1]

[1]*Centro Hospitalar Médio Ave (CHMA)*

Introduction: Leukemias are a rare type of neoplasms. They comprise a heterogeneous group of entities, with different clinicopathological characteristics. They may appear in pediatric age and present an aggressive clinical course.
With the development of diagnostic techniques, from the determination of the blood count, the morphological study of peripheral blood, immunophenotyping, cytogenetics and molecular biology, as well as the different therapeutic options, the rates of early detection and survival have increased.

Clinical Case: Male child, 12 years old, with no relevant personal history, seeks medical attention at the emergency department with marked asthenia, fever, dyspnea, nausea and vomiting. Upon clinical observation, he was conscious and oriented, with mucocutaneous pallor and poor peripheral perfusion. Analytically it was observed anemia (hemoglobin 3,2 g/dl), leukocytosis ($47.17x10^3$/mL), lymphocytosis (53% of lymphocytes; $25.00x10^3$/mL) and elevation of the enzyme Lactate Dehydrogenase (2349 UI/L). In the determination of the blood count, using the Sysmex XN-1000 equipment, it was possible to visualize changes in the leukocyte scatter plot and alerts were issued by the equipment for the possible presence of blasts/atypical cells. A peripheral blood smear was performed, which showed marked lymphocytosis and the presence of about 34% of blasts.
In the immunophenotyping study it was revealed the presence of 72% of blasts with meyloid and B lymphoid immunophenotype, compatible with Acute Mixed B/Myeloid Leukemia (immunophenotype: CD3-, CD7-, CD13-, CD14-, CD19+, CD20-, CD24+, CD33-, CD34-, CD45+, CD58+, CD64-, CD81+, CD117-, CD123+, MPO+. DNA Index: 1.34; Ploidy: Hyperdiploid). A cytogenetic study of peripheral blood was performed, identifying the presence of a structural change on chromosome 14 (14q32, location of the IGH locus); a bone marrow sample was also studied, verifying the presence of the alteration in chromosome 14, and the presence of a deletion in the CDKN2A gene (9p21).

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

P12

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

Ana Macêdo[1], Dialina Brilhante[1], Filipe Gonçalves[1]

[1]*SIH, IPO Lisboa*

The study of hemolytic anemia represents a challenging endeavor to the immunohematology labs. Specific methodologies should be available to circumvent this fact.
We describe 3 clinical cases of Warm Autoimmune Hemolytic Anemia (WAIHA), Cold Autoimmune hemolytic Anemia (CAIHA) and Drug induced hemolytic anemia (DIIHA) in the clinical perspective and serological approach.

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

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

Case 3 (DIIHA): Female, diagnosis of germinal cell tumor of the hypophysis/optical quiasma, in 2002. CR after surgery + high dose chemotherapy and RT. Relapse in 2013 and 2º relapse in 2019 after AutoSCT. She was admitted in the ICU for sepsis from CVC and empirical antibiotherapy was started with Piperacilin/Tazobactam and vancomycin. Her condition worsens, with severe anemia (Hb 4,9 g/dL) and hemolysis. 2 units of pRBC were requested to the blood bank. Positive DAT and IAT with eluate negative. X-match positive. Coke - colored like plasma. Serological studies evolving drugs conducted to the suspension of Piperacillin/Tazobactam intake.
Exclude the presence of alloantibodies, not delay transfusion of RBCs and organize a SOP for the laboratory study of AIHA.

P13

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

Sara Queirós[1], Filipe Pinto[2], Catarina Faria Coelho[1], Luciana Xavier[2], Cristina Gonçalves[2], Maria dos Anjos Teixeira[3], C Iracema Morais[4], Lídia Branco[5]

[1]*Laboratory Hematology Service, Department of Clinical Pathology, Porto University Hospital Center, Porto, Portugal*
[2]*Department of Clinical Hematology, Porto University Hospital Center, Porto, Portugal*
[3]*Cytometry Laboratory, Department of Clinical Hematology, Porto University Hospital Center, Porto, Portugal; Unit of Multidisciplinary Research in Biomedicine, ICBAS – Abel Salazar Institute of Biomedical Sciences, Porto University, Porto, Portugal*
[4]*Immunology Service, Department of Clinical Pathology, Porto University Hospital Center, Porto, Portugal; Unit of Multidisciplinary Research in Biomedicine, ICBAS – Abel Salazar Institute of Biomedical Sciences, Porto University, Porto, Portugal*
[5]*Immunology Service, Department of Clinical Pathology, Porto University Hospital Center, Porto, Portugal*

Introduction: Plasma Cell Leukemia (PCL) is a rare and aggressive variant form of myeloma defined by the presence of peripheral blood plasmacytosis, accounting for >20% of the differential or >2x10^9/L of circulating plasma cells. In Europe, the incidence is 0.4 per million individuals per year. The prognosis is poor, with median survival below one year.

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

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

P14

FLAGGING PERFORMANCE EVALUATION OF THE BECKMAN-COULTER DXH900 HEMATOLOGY ANALYZER

Daniel Nunes[1], Camila Saraiva[1], Marta Manaças[1], Cristina Duarte[1], Filipa Fernandes[1]

[1]*Hospital de Santa Maria, CHULN*

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

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

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

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

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

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

P15

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

Ana Macêdo[1], Dialina Brilhante[1], Mario Cunha[2], Zélia Videira[2]

[1]*SIH, IPO Lisboa*
[2]*SPC, IPO Lisboa*

The implementation of a transfusional security system was mandatory for Blood Banks and Transfusional Medicine Services. In our hospital we have adopted BTrac solution. These system includes the collection of a main sample for crossmatch at bedside, identifying the patient using the wristband adopted in the hospital, and the application of transfusion identifying who performs the application, the vital signs and times of infusion. In our hospital we have adopted these solution and also the use of a routine sample for Complete Blood Count, because our patients performed them in daily basis. Thus we have two pathways to obtain samples that can be used to perform crossmatch if the patient needs transfusional support.

In order to use these sample of CBC an interface between BTrac and Clinidata was done and all the samples for analytical routine can be collected at bedside.
For achieving this endeavor, we organize, in collaboration of Pathology Laboratory team, all the pre-analytical phase in wards: collection of different samples at the beside of the patients, using the wristband, identifying the nurse and the time of collection of these samples. And we decided to implement LabTrac.

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

This solution is applied to in ward patients and outward patients with the exception of the Central place of sampling.
The main values we won were the standardization of all the collections, the organization of time of collection by nurses, very important for in ward patients, the use of patient wristband identification as the starting point for these pathway, and last but not the least safety for patients and personal.

P16

BURKITT´S LYMPHOMA - THE ROLE OF THE CLINICAL PATHOLOGIST

Sara Moura[1], Sílvia Freitas Arroja[2], Ana Maria Meireles[3], Cláudia Moreira[3], Raquel Coentrão[3], Teresa Sousa[3], Carlos Mendes[3], Paula Pinto[1]

[1]*Hospital Distrital de Santarém*
[2]*Centro Hospitalar Vila Nova de Gaia e Espinho*
[3]*Instituto Português de Oncologia do Porto Francisco Gentil*

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

Sporadic BL is seen mainly in children and young adults, but there is also an incidence peak in elderly patients; more prevalent in the male.
BL is a highly aggressive but potentially curable tumour, with long-term overall survival in 70-90% of cases. However, there are several adverse prognostic factors.
We report a case of sporadic form of BL.

Clinical case: A 84-year-old man with medical history of heart failure, primary cutaneous follicular NHL, diagnosed in 2013 with complete response, and prostate adenocarcinoma.
Patient reports a need for help in activities of daily living in recent months; associated with anorexia, insomnia, headache and lumbar back pain.
Laboratory findings revealed anemia, leukocytosis of 96730/uL (neutrophils 67230/uL, lymphocytes 4840/uL, monocytes 2420/uL) and thrombocytopenia of 58,000/uL; elevation of LDH, uric acid, ferritin and CRP.
The peripheral blood smear showed a shift to the left of the granulocytic series with 1.5% of blasts and the presence of erythroblasts.
The myelogram reported hypercellular marrow, reduced number of megakaryocytes; presence of 31% of large-sized cells, large nucleus/cytoplasm ratio and hyperbasophilic cytoplasm with vacuoles-"starry-sky" appearance.
The histology describes as hypercellular with diffuse pattern lymphoid infiltrate, consisting of intermediate to large sized cells with irregular nuclei and evident nucleolus: CD20+ CD3- CD34- TdT- MPO- CD117- Cam5.2-.
Flow cytometry confirmed the presence of 21% of blasts with CD5- CD10+ CD19+ CD20- CD34- CD38+/++ CD43+ CD79b-/+weak CD200-/+weak cKappa+ TdT- immunophenotype. Hyperdiploid with a high proliferative index of 62%.
Cytogenetics identified the t(8;14)(q24;q32) involving the MYC and IGH genes.
The patient died at home while awaiting laboratory results.

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

P18

INFECTIVE ENDOCARDITIS BY ABIOTROPHIA DEFECTIVA: A CASE REPORT

José João Flores dos Santos[1], Dra. Carla Leite[1], Dr. Fernando Fonseca[1]

[1]*Centro Hospitalar Póvoa de Varzim/Vila do Conde*

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

This case describes an 85-year-old man sent to the emergency department after blood work in a telemedicine appointment revealed anaemia. The patient presented with fever of unknown origin. Relevant medical history included a prosthetic aortic valve, type II Diabetes mellitus and a recent ischemic stroke.
Blood and urine cultures were made. A gram-positive coccus was detected in all three blood cultures. Identification was done with an automated Vitek 2 Compact device, which yielded a presumptive diagnosis of *Abiotrophia defectiva*. This result was confirmed by an external laboratory.

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

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

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

P19

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

Amadeu Gomes[1], Clara Barros[1], Sérgio Neves[1], Telma Dias[1], Ana Pereira[1], Márcia Neto[1], Maria Couto[1], Sónia Carvalho[1], Olinda Dias[1], Valquíria Alves[1], Maria Soares[1], Eugénio Corrêa[2], Teresa Silva[2], Cidália Coelho[2]

[1]*Unidade Local de Saúde de Matosinhos – Hospital Pedro Hispano*
[2]*Laboratório Synlab Porto*

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

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

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

Results: Overall, of 1951 patients, 1651 (84,6%) were female (major prevalence over the age 80-89 years) and 300 (15,4%) were male (major prevalence 70-79 years of age).
Of the 1951 *E. coli* strains tested 4.5% were ESBL producers, 99.4% (n=1939) showed good agreement between the two methods. Discrepant results were obtained in 0.62% (n=12). Of these, 4 were resistant by Vitek®2 AST-355 and were confirmed by E-test. The remaining 8 were susceptible to fosfomycin by Vitek®2 AST-355 and resistant by E-test, corresponding to 0.41% of discordant results. In this study Vitek®2 AST-355 method had a sensitivity of 99.6% and a specificity of 100%.

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

P20

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

Francisco Freitas[1], Ana Simões[1], Isabel Vale[1], Luísa Frias[1], Maria Pires[1], Maria Tomás[1], Marco Lopes[1], Jorge Loureiro[1]

[1]*Laboratório de Microbiologia, Centro Hospitalar Tondela-Viseu*

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

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

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

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

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

P21

MICROSPORUM CANIS INFECTION IN A PAEDIATRIC PATIENT: A CASE REPORT

Luís Morais[1], Rui Figueiredo[1], Rosa Soares[1], Paula Gama[1], Carlos Cortes[1]

[1]*Centro Hospitalar do Médio Tejo*

Keywords: *Microsporum canis*; dermatophytes; paediatric

Introduction: *Microsporum canis* is a zoophilic dermatophyte with cats and dogs as natural hosts. *M. canis* is highly contagious, easily transmitted to humans, causing glabrous skin (*tinea corporis*) and head (*tinea capitis*) infections. *Tinea capitis* (TC) is a common cutaneous fungal infection among 2 to 7 year old children but rare in the first year of life.
In this present case, cultures were performed using SGC2 agar to identify dermatophytes. Plates were incubated at 25 °C and examined every 2–3 days along 15 days. Identification was based on macroscopic and microscopic observation. Macroscopic examination revealed some white fluffy spreading colonies with a characteristic deep yellow-orange pigment reverse. "Spindle" shaped multicellular macroconidia with thick cell walls were observed on microscopic examination. Clinical features and culture results revealed a *M. canis* TC.

Case presentation: We present a case of a 2-year-old and a 1-year-old siblings presenting erythematous scalp lesions combined with 20 cm diameter extensive alopecia. Suspecting dermatophytosis a mycological analysis of all lesions was performed. Clinical features, direct examination, and culture results confirmed tinea capitis caused by *M. canis.*
Itraconazole (an *imidazole*/triazole type) has been the antifungal treatment of choice.

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

P22

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

Luís Santos[1], Alicia Rodrigues[1], Olga Costa[1], Irene Carrapatoso[1], Madalena Almeida Santos[1], Helena Matias[1], Lídia Santos[1], Lurdes Lopes[1], Patrícia Miguel[1], Paula Baranito[1], Paula Branquinho[1], Sara Gomes[1], Sofia Santos[1], Teresa Ferreira[1], Rita Côrte-Real[1]

[1]*Serviço de Patologia Clínica, Centro Hospitalar Universitário Lisboa Central*

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

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

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

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

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

P23

NOCARDIA BRASILIENSIS ISOLATED IN AN ELBOW WOUND ABSCESS

João Pedro Porto[1], Carolina Salteiro[1], José Carvalho Marta[2]

[1]Centro Hospitalar e Universitário de Lisboa Central, [2]Centro Hospitalar e ~universitário de Lisboa Central

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

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

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

P24

BCGITIS IN AN END STAGE RENAL DISEASE PATIENT

Diogo Silva[1], Teresa Almeida[1], Teresa Carvalho[1]

[1]*Serviço de Patologia Clínica do Centro Hospitalar Universitário de São João*

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

Case report: A 74 year old male with multiple comorbidities, including stage 5 Chronic Kidney Disease (CKD) and high risk non-muscle invasive bladder cancer treated with BCG intravesical immunotherapy, presented to the ER with generalized pain, foul smelling urinary discharge and urgency (patient was usually anuric) and fever. The type II urine test revealed leukoerythrocyturia, resulting in a diagnosis of a urinary tract infection (UTI) and the patient being medicated with cefixime.
A week later, the patient was returned to the ER, after a hemodialysis session, in a confused state, maintaining urinary symptoms and fever. Inflammatory markers were elevated and type II urine test was unable to be performed due to macroscopic pyuria. The diagnosis of a cefixime resistant UTI was assumed, the antibiotic was changed to piperacillin/tazobactam and the patient was admitted to an Internal Medicine Ward. Urine and blood cultures were negative. 3 days later, after absence of improvement, micobacteriological urine smear and culture were ordered. The smear showed numerous Acid-Fast Bacilli (AFB) and the culture was positive for cord forming AFB, which were identified by GenoType MTBC kit to be BCG. The patient was diagnosed with bladder BCGitis and was transferred to the Infectious Diseases Ward were he was treated with a HRE regimen and showed symptomatic improvement.

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

P25

CLINICAL INFORMATION: THE MISSING PIECE TO URINE CULTURE PUZZLE?

Gonçalo Torres Pinto dos Santos[1], Hermínia Marques[1], Paula Mota[1]

[1]*Serviço de Patologia Clínica do Hospital da Senhora da Oliveira - Guimarães*

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

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

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

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

P26

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

Antonio Eduardo Figueiredo[1], Maria Luis Guerra[1], Lúcia Coimbra[1], Ana Raquel Loureiro[1], Ângela Dias[1], Nazaré Boavida[1], Luísa Sancho[1]

[1]*Hospital Prof. Doutor Fernando Fonseca, EPE*

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

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

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

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

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

P27

URINARY TRACT INFECTION IN A COMMUNITY LABORATORY

Ana Catarina Faria Guerreiro[1], João Lago[1], Renato Lourenço[1], Gizela Santos[1]

[1]*Laboratório análises clínicas Dr J Leitão Santos*

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

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

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

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

P28

LABORATORY TESTS FOR SARS-COV-2 SCREENING

Alicia Rodrigues[1], Luis Santos[1], Irene Carrapatoso[1]

[1]*CHULC*

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

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

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

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

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

P29

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

Svetlana Zhelezovskaya[1], Rita Francisco[1], Cláudia Reynolds[1], Pedro Gouveia[1], Maria Matos Silva[1], Joaquim Freitas[1], Maria Callé[1]

[1]*Centro Hospitalar Tâmega e Sousa*

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

Objectives: Taking into account the same mode of transmission of EBV and SARS-Cov-2 viruses by airborne route and droplets, the authors intended to analyze retrospectively the evolution of EBV incidence during three autumn-winter periods 2019-2022.
Simultaneously, they want to evaluate the influence of the public health measures taken to counteract the SARS-Cov-2 pandemic on EBV transmission.

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

Results: Regarding the age group, the highest incidence was recorded in the group from 0 to 19 years old. A reduction of the total number of requests in autumn-winter 2020/2021 was found, although the percentage of positive results remained constant across the years, 9%-11%.
The majority of cases (53%-100%) was detected in the Emergency Department, during the three periods of the study.

Conclusion/Discussion: The reduction of total number the request in autumn-winter 2020/2021 can be due to the reduction of Emergency Department attendances during the third SARS-Cov-2 wave. On the other hand, the percentage of positive tests remain constant along the studied period, with a mean value of 10%. Taking into account the common mode of transmission and the generalized use of facemasks and other preventive measures against SARS-Cov-2, EBV infection might have presented a decrease, yet this was not observed.
It is well known that the age range of greater EBV risk (0 to 19 years), and especially pre-school children, had less adherence to social distancing measures and to the use of masks. This might, at least, in part, explain the obtained results.

P30

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

Cristiana Isabel Oliveira Brito Pires Lopes[1], Claúdia Fernandes[1], Margarida Peixinho[1], Eulália Costa[1], Fernando Rodrigues[1]

[1]CHUC

Introduction: Vitamin C (VitC) or L-ascorbic acid is a water-soluble antioxidant vitamin obtained only through diet or supplementation.
Vitamin C is essential to many abolic pathways, is very unstable in vitro, rapidly oxidizing to dehydroascorbate. This oxidation process is due to pH, high temperature and presence of oxidizing enzymes or iron ions, decreasing the concentration of VitC.
Studies indicate that tris [2-carboxy-ethyl] phosphine hydrochloride (TCEP) can slow down this oxidation process. Pre-analytical stability assessment is essential to define maximum storage time while maintaining sample stability.

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

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

Results: Mean bias variation of samples without stabilizer was: - 14.6% (T0-T1), -48.8%, (T0-T2), -77.5% (T0-T3), -85.1% (T0 -T4) that correspond an average decrease of 11.77 mg/l in the VitC sample.
Mean bias variation of samples with stabilizer was: -5.9% (T0-T1), -20.4%, (T0-T2), -24,5% (T0-T3), -28,6% (T0 -T4) that correspond an average decrease of 4.15 mg/l in the VitC sample.
Mean bias variation of intra-sample (with and without TCEP) in the same run was: -4.6% (T0), -11.9% (T1), -37.8% (T2), -72.2% (T3) and -80.5% (T4).

Conclusion: Despite the small sample of this study, we realized that the use of TCEP is essential to improve the laboratory pre-analitical time, to ensure the best practices for measuring Vit C.
Without TCEP, samples can be frozen for up to 7 days without clinical impact. Using the stabilizer this time increases up to 21 days with a bias of 28.5%, however acceptable in this methodology. The use of TCEP improves the laboratory pre-analytical workflow at least 21 days.

P31

DIRECT AND INDIRECT ISE POTENTIOMETRY AND PSEUDOHYPONATREMIA - A CLINICAL CASE

Bernardo Ribeiro da Silva[1], Carlos Miguel Oliveira[2], Nuno Gonçalves[2]

[1]*Centro Hospitalar de Leiria, E.P.E.*
[2]*Instituto Português de Oncologia do Porto Francisco Gentil E.P.E.*

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

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

Discussion: Pseudohyponatremia is a common laboratory abnormality defined by a Na^+ concentration lower than 135 mmol/L in the presence of an isoosmolality. Common causes include hyperlipidemic or hiperproteinemic conditions such as monoclonal gammopathy malignancies, amyloidosis and intravenous immunoglobulin therapies. It occurs in IP due to the dilution of the sample (total serum), which aims to reduce ionic interference. In this case, we observed a patient with an increased total serum proteins (and as such, an increase in the solid phase). Since the dilution volume is fixed, it does not take in consideration changes in the water-solid phases proportions, so an increase in the solid phase leads to a falsely decreased Na^+ value. The measurement by DP allows a more accurate result as it does not suffer interference from lipids and proteins.
The perception by the Clinical Pathologist is crucial for the early detection of this error and avoiding undue treatment (as reported in literature[1]) and increased costs. Thus, laboratory communication with the clinical team is crucial for the correct diagnosis and treatment of the patient.

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P32

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

Cristiana Lopes[1], Teresa Reis[1], Joana Oliveira-Silva[1], Ricardo Batista-Silva2[1], Ricardo Ribeiro[2], Sabina Brito[1], Fátima Amado[1], Lucília Araújo[1], Isabel Antunes[1], Fernando Rodrigues[1]

[1]CHUC
[2]CHUP

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

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

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

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

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

Results: At T0, 100% (n=1673) of participants enrolled were naïve and had non-reactive IgG antibodies to SARS-CoV-2. Fifteen days after completing the vaccination, the IgG overall median titer was significantly elevated (21.3 x10^3 AU/ml). Comparing data with T1 results we observed a waning of antibody titers throught time: a decline of 6.6 fold (-84.8%) at T2; 20.36 fold (-95.1%) at T3; 36.1 fold (-97.2%) at T4. At T5, after 3th dose, a rise of 0.71 fold (+40.56%) compared with T1.

Conclusions: After vaccination with the BNT162b2 vaccine, anti-SARS-CoV-2 IgG kinetics tend to peak around 14 to 30 days, followed by a substantial reduction over time, with significantly lower levels at 10-months as described in similar studies. Also the rise of antibodies after booster, above those found in T1, is described elsewhere but the long-term durability of such protection remains to be determined.
These findings support the need to track humoral immunity kinetics to uncover viral susceptibility and eventually implement re-vaccination, particularly in groups prone to lower humoral immune response.

P33

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

Jorge Manuel Vale Pinheiro[1], Alvim A.[1], Anastácio M.[1], Costa N.[1], Cardeira S.[1], Camara A.[1], Carpalhoso B.[1], Carriço A.[1], Carvalho A.[1], Cunha R.[1], Ervilha C.[1], Ferreira F.[1], Figueiredo M.[1], Gomes D.[1], Guerra A:[1], Lopes C.[1], Louro N.[1], Luís L.[1], Marrão G.[1], Mendes S.[1], Menezes M.[1], Morgado D.[1], Oliveira A.[1], Oliveira C.[1], Órfão D.[1], Pedrosa J.[1], Pires Letícia[1], Ribeiro Y.[1], Roda S.[1], Santos L.[1], Santos H[1], Santos M.[1], Silva F[1], Simões Isabel[1], Simoes Inês[1], Vieira L.[1], Vieira R.[1], Silva C.[1], Silva S.[1], Sudor Y.[1], Paquim A.[1], Ostapenko N.[1], Castro Ricardo[1]

[1]Pathology Service of Centro Hospitalar de Leiria EPE

Introduction: Reference Change Value (RCV) is one of the main indicators of the clinical quality of a dosing method for personalized patient monitoring. Its calculation involves the use of intra-individual biological variation data and long-term analytical CV (CVa) (1,2). The highest CVa of the less precise internal quality control level can be used to calculate the RCV for all the different concentration or activity of a dosing method.
Depending on the method precision, we can have more than 30% of patient results with analytical errors beyond the highest CVa.
A practical way to estimate the percent number of patients results (PR) beyond the CVa is to use the laboratory information system (LIS) to calculate the percent of controls beyond the highest CVa (CTLcv).
If the PR beyond a CVa is unacceptable, we can calculate the PR under the error of the extended uncertainty measurement (2xCVa), that usually includes 100% of PR.

Material and Methods: We use the Modulab Gold LIS to evaluate the CTLcv of the last 6 months that have errors above and under +-1 sd for the highest CVa observed on the quality control charts for potassium (K), total cholesterol (Col) and thyroxine-free (FT4).
We calculate the RCV using the online calculator at EFLM Biological Variation Database (2), for 95% probability.

Results: The CTLcv estimated for the highest CVa were: K= 21.6%; Col=32.8% ; FT4= 23.8%
The RCV calculated for the highest CVa : RCVK= 11.8% ; RCVCol=15.4% ; RCVFT4= 24.5%
The RCV calculated for the expanded uncertainty of measurement (2xCVa) for the highest CVa and 0% CTLcv: RCVK= 12.9% ; RCVCol=17.3% ; RCVFT4= 43.1%.

Conclusion: We can use RCVK=11.8% for only 78.4% (100-21.6) of all of K results for monitoring patients, but with RCVK=12.9% we can use it for monitoring all of K patient results.
We can use RCVCol=15.4% for only 67.2% (100-32.8) of all of Col results for monitoring patients, but with RCVK=17.3% we can use it for monitoring all of Col patient results.
We can use RCVFT4=24.5% for only 76.2% (100-23.8) of all of K results for monitoring patients, but with RCVK=43.1% we can use it for monitoring all of FT4 patient results.
When we use CVa that CTLcv=0%, on RCV calculations, it include the analytical error of all measurements and therefore adequate for monitoring all patients.

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P34

AN UNEXPECTED CAUSE OF HYPERTHYROIDISM

Silvia Freitas Arroja[1], Jorge Meneses[1], Cátia Santa[1], Lino Azevedo[1], Ana Paula Cruz[1]

[1]*Centro Hospitalar de Vila Nova de Gaia e Espinho, EPE*

Introduction: Hyperthyroidism has different etiologies. Graves' disease (GD) is an autoimmune disorder caused by antibodies targeting the thyrotropin receptor of the thyroid cells (TRAb), leading to an increased production of thyroid hormones and inducing an inflammatory process in the thyroid. Endocrine orbitopathy is often present in GD and some patients develop exophthalmos. In patients with GD orbitopathy there may be additional antibodies directed against the insulin-like growth factor type 1

receptor (IGF-1R) which is overexpressed on the orbital fibroblast. Another aetiology for hyperthyroidism is toxic adenoma, a single hyperactive autonomously functioning thyroid nodule that produces supraphysiological amounts of T4 and/or T3 resulting in suppression of serum thyroid stimulating hormone (TSH). The coexistence of GD and thyroid functioning nodules is rare and is called Marine-Lenhart syndrome, estimated to occur in 0.8-2.7% of patients with Graves' disease.

Case Presentation: We present the case of a 53 year old female referred for abnormal thyroid function test. Patient reported hair loss, palpitations and fatigue but denied any difficulty swallowing, bowel habits, weight loss, radiation to neck, new medications or family history of thyroid disease. Laboratory tests showed TSH <0.01 (0.27 - 4.2 uIU/mL), free T3 15.12 (2.57 - 4.43 pg/mL), free T4 3.5 (0.93 - 1.7 ng/dL) and anti-TSH of 27,0 IU/L (<1.75 IU/L). Thyroid ultrasound showed a right thyroid lobe nodule measuring 44 mm. Thyroid scintigraphy showed an intranodular distribution of 99mTc-pertechnatate consisting of an autonomously functioning thyroid nodule in the right lobe of the thyroid. The patient was treated with methimazole followed by thyroidectomy and four years after initial diagnosis TRAb´s were still present and patient had to undergo bilateral orbital decompression followed by bilateral blepharoplasty.
Discussion: Marine-Lenhart syndrome is a rare cause of hyperthyroidism with diagnosis criteria still not well established. Although hyperthyroidim caused by two different processes occurring in the same patient is a rare condition, it is important to have a high índex of suspicion when evaluating thyroid disease and perform complete thyroid function tests.

P35

A LABORATORY LOOK IN TO WILSON´S DISEASE: A TEN YEARS RETROSPECTIVE STUDY

Margarida Peixinho[1], Eulália Costa[1], Anália Carmo[1], Ângela Maresch[1], José Carvalho[1], Paula Caseiro[1], Fernando Rodrigues[1]

[1]CHUC

Introduction: Wilson's disease is a rare and progressive autosomal recessive disorder in which changes in copper (Cu) metabolism result from the presence of mutations in the ATP7B gene. These changes lead to Cu deposition in different organs and clinical manifestations are directly related to the sites of excessive Cu accumulation, being liver failure and dysfunction of central nervous system the most frequent findings. Early diagnosis, initiation of therapy and monitoring of urinary (U) and serum (S) Cu levels are essential.

Objective: Evaluation of laboratory parameters of individuals with Wilson's disease, in a tertiary hospital.

Methods: Retrospective study of 25 patients diagnosed with Wilson's disease, for 10 years. Parameterized research using the laboratory's computer system to analyze the CuU, CuS and ceruloplasmin (Ce) values of these patients. Assessment and study of the following variables: age, gender, clinical presentation, laboratory tests at diagnosis, liver biopsies, rhodamine staining, liver Cu assay and presence of Kayser-Fleischer rings.

Results: The age of the patients ranged from 11 years to 69 years (33.6 ± 16.2 years), 5 in pediatric age, being 13 males and 12 females. Three (12%) had a family history of Wilson's Disease, 6 (24%) had no family history and 16 (64%) had no clinical information available. Cu in liver biopsy was determined in two patients, with an average concentration of 1018.5± ug/g. At the time of diagnosis, the mean CuS was 0.048±0.606mg/L, the CuU measurement was 0.471 ±0.298, and the Ce was 0.075±0.058g/L. Rhodamine staining was negative for all patients. In 28% of the cases, liver cirrhosis was the presentation of the disease, with ascites being present in 4% of the total. Only 4% had Kayser-Fleischer rings. From the genetic study carried out, it was found that 48% had mutations in the ATP7B gene, of which 20% had variants in homozygosity and 28% in heterozygosity.

Conclusion: The patients presented a significant reduction in Ce and in CuS and an increase in the CuU, with liver cirrhosis being the most frequent presentation. Avoiding deposition of Cu in tissues is fundamental in this life treatment disease, showing that laboratory determination of CuU/S and Ce is crucial to monitoring the adequacy of therapy.

P36

COMPARATIVE STUDY OF TWO AUTOMATED IMMUNOASSAYS FOR THE DETERMINATION OF NEURON-SPECIFIC ENOLASE

Luís Roseta[1], Sabina Silva[1], Fátima Amado[1], Ângela Maresch[1], Eulália Costa[1], Fernando Rodrigues[1]

[1]*Centro Hospitalar e Universitário de Coimbra*

Introduction: Neuron-Specific Enolase (NSE) is a cell-specific isoenzyme of enolase found in neuronal and neuroendocrine tissues. As a tumor marker, it can help support the diagnosis of small cell lung cancer, contributing to its monitoring and prognostic stratification, as well as for other neoplastic and nonmalignant diseases.
Nowadays, automation is fundamental to optimize the management of workflow and resources. When choosing an immunoassay, quality of the results is of paramount importance.

Objective: Evaluate the performance of two immunoassays to determine the concentration of NSE in serum samples.

Materials and methods: Over 5 months, 163 samples were sequentially processed in KRYPTOR® compact PLUS (BRAHMS®) (KB) – TRACE method – and in Alinity i® (Abbott®) (AA) – immunoassay microparticles by chemiluminescence; both according to manufacturer's instructions. Hemolyzed samples were excluded.
Manufacturer's reference values (RV) were: <12,7 ng/mL (KB) and <11,1 ng/mL (AA). Statistical analysis was performed using Microsoft Excel® software, including the determination of Pearson's correlation coefficient.

Results: Concentration ranges varied between 6,19–69,97 ng/mL (KB) and 4,0–57,9 ng/mL (AA).
Comparison between the two methods showed a strong positive correlation (y=0,9615x-3,9471; R^2=0,94). The mean concentration of NSE was 16,68 ± 8,8 ng/mL (KB) and 12,10 ± 8,8 ng/mL (AA) (mean ± standard deviation (SD)). The mean difference between pairs of results was 4,59 ± 2,2 ng/mL. The mean bias was -30,67% with a variability of 12,7%.
With respect to samples exceeding RV, 114 samples were above RV after being analyzed in KB contrasting with 62 samples in AA, corresponding to 31,9% of potential discrepant clinical decisions, with the following means and SD: 19,5 ± 9,1 ng/mL (KB) and 18,4 ± 10,8 ng/mL (AA). Of notice, the mean difference between pairs of results was of 4,72 ng/mL (minimum 0.58 ng/mL, maximum 12,07 ng/mL).

Conclusion: The concentration of NSE may support diagnosis, monitoring and prognosis of several diseases. The results obtained through the two methods revealed a good correlation, so both could be valuable options for laboratory automation for NSE determination.

P37

IMMUNE RESPONSE TO MRNA COVID-19 VACCINATION – A LONG TERM STUDY IN HEALTH CARE PROFESSIONALS

Carlos Caldas[1], Joana Pina[1], Maria Martins[1], Marisa castro[1], Rafael Vilamarim[1], Vera Ribeiro[1], Eliana Costa[1]

[1]*CHTMAD*

In December 2019, SARS-CoV-2 was identified in city of Wuhan, China. It is a causative agent of an acute respiratory disease, coronavirus disease 2019[1]. Vaccines to prevent SARS-CoV-2 infection are considered the most promising approach for curbing the pandemic and the global strategy to control COVID-19[2]. SARS-CoV-2 antibodies are less useful in treating the disease but will play an important role in determining and monitoring the immunity levels as the epidemic progresses[3]. The information provided by systematic hospital-based screenings can contribute to a knowledge gap on SARS-CoV-2 circulation in the general population[4,5].

Our aim was evaluating the SARS-CoV-2 seroprevalence in our Hospital Center healthcare professionals (HCP), after Pfizer-BioNTech COVID-19 vaccination.

The Administrative Council approved a prospective study to evaluate the immune response after vaccine intake among HCP. A total of 1296 individuals (1011 female and 285 male) who were vaccinated and met all the criteria for this study, (absence of IgG (anti-RBD/S) antibodies prior to vaccination and monitoring of levels of each during approximately ten months) were selected. A representative sample of the data collected, was chosen and 440 CHTMAD HCP (340 females and 100 male), blood samples, that had been harvested before vaccination (T0) and after one (T1), three (T2), six (T3) and nine (T4) months after second dose vaccine uptake were analysed. The levels of antibodies were determined by a chemiluminescent microparticle immunoassay (AdviseDx SARS-CoV-2 IgG II, Abbott Diagnostics, Abbott Park, IL, USA), designed to detect IgG antibodies to the receptor binding domain (RBD) of the S1 subunit of the spike proteins of SARS-CoV-2. Results were analysed using GraphPad prism Software 6.0 and t-student test for statistical analysis.

The level of IgG (anti-RBD/S) antibodies decreased significantly over time ($p < 0,0001$), and no significant differences in antibody titers were observed by gender. When we compare the level of antibodies we could see the levels decreasing as the age increases (female: slope -197.5 to 54.05; male: slope -279.8 to 2.64). The anti-spike IgG level was substantially lower nine months after vaccination (T4). This data supports the need of a third dose of mRNA vaccine.

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P38

EVALUATION OF TWO AUTOMATED IMMUNOASSAYS FOR THYROGLOBULIN ASSAY: ARE ALL ASSAYS CREATED EQUAL?

Luís Roseta[1], Fátima Amado[1], Sandra Vitor[1], Sabina Silva[1], Ângela Maresch[1], Eulália Costa[1], Fernando Rodrigues[1]

[1]*Centro Hospitalar e Universitário de Coimbra*

Introduction: Thyroglobulin (Tg), synthesized in thyroid follicular cells, is the most expressed protein in the thyroid gland. The main application of this tumor marker is postoperative follow-up of patients with differentiated thyroid carcinoma, however it can also help in the diagnosis of Hashimoto's disease, Graves' disease, thyroid adenoma and carcinoma, amongst other diseases.

Tg determination is generally performed through immunoassays (IA). As multiple automation options are available, choice must take into account quality of the results, the target population and workflow of the laboratory.

Objective: This study aims to compare two IA to determine the concentration of Tg in serum samples.

Materials and methods: Over 5 months, 171 samples were processed sequentially in Cobas® e411 (Roche®) (CR) – electrochemiluminescence – and Alinity i® (Abbott®) (AA) – chemiluminescence microparticle immunoassay; both according to manufacturer's instructions.
Manufacturer's reference intervals (RI) were: 3,50-77,00 ng/mL (CR) and 3,68-64,15 ng/mL (AA). The lower limit of detection (LoD) were 0,04 ng/mL and 0,09 ng/mL for CR and AA respectively. Statistical analysis was performed using the software Microsoft Excel®, including the determination of the Pearson correlation coefficient.

Results: Concentration ranges varied between 0,04–341,90 ng/mL (KB) and 0,09–378,92 ng/mL (AA).
The two IA showed a strong positive correlation ($y=1,0962x + 0,3867$; $R^2=0,9957$). Mean Tg concentration was 14,22 ng/mL (CR) and 15,98 ng/mL (AA). Mean difference between the results pairs was -1,75 ± 5,3 ng/mL.
Considering the RI, 124 (72,5%) samples showed Tg concentrations outside the RI in the CR, while in the AA there were 122 (71,3%), demonstrating 1,2% of potential discrepancy in the clinical decision.

Conclusion: Tg concentrations are essential in the diagnosis and follow-up of thyroid disorders.
The results of the two methods revealed an excellent correlation, with only 1,2% of discrepancy in clinical decision. The LoD differs between the equipments, the impact of which should be evaluated for the thyroidectomized patients. Our study showed that both the IA could be valid automated alternatives for clinical laboratories.

P39

APPLICATION OF BIO-RAD D-100™ SYSTEM ANALYSER FOR HBA1C MEASUREMENT AND THE HEMOGLOBIN VARIANTS SCREENING

Ana Isabel Pereira Fernandes Rodrigues[1], Ana Cristina Marques[1], Carla Oliveira[1], Maria João Lopes[1], Sílvia Rodrigues[1], Eulália Costa[1], Fernando Rodrigues[1], Teresa Reis[1]

[1]*Centro Hospitalar e Universitário de Coimbra*

Introdution: Diabetes mellitus is characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy, and affects approximately 7% of the world's population. HbA1c is the stable glucose adduct to the N-terminal group of the beta-chain of HbAo. The results of HbA1c can be used either for diagnosis or monitoring the glycaemic state in diabetic patients, with a frequency of two to three months interval, in human blood. The Bio-Rad D-100™ system is a fully automatic benchtop analyzer for determination of HbA1c, based on high performance liquid chromatography (HPLC), this equipment detects normal and abnormal peaks, such as hemoglobin variants. When a possible variant is detected, we reprocess the sample in the SEBIA MINICAP FLEX PIERCING equipment (Capillary electrophoresis), for confirmation. Analytical Interferences in HbA1c assays: red blood cell half-life; carbamylated hemoglobin; acetylated hemoglobin; Heterozygous hemoglobinopathies (variants S, F, C); Homozygous hemoglobinopathies (absence of HbA).

Aim: Evaluation of HbA1c values from a pool of samples, using Bio-Rad D-100™ system and the casuistic of the hemoglobin variants detected.

Materials and Methods: A retrospective study of HbA1c assays performed in the Bio-Rad D-100™ equipment and statistical evaluation of the presence of variants, after confirmation in the SEBIA capillary electrophoresis equipment. Data were analyzed using Microsoft Excel software.

Results: retrospective study of 13604 random samples, where the average age was 55.9 (± 17.4). For 6 months, the HbA1c value was calculated in all samples using the Bio-Rad D-100™ system and from these, 45 (0.33%) hemoglobin variants were found. HbS was detected in 32 samples (71.1%) and also the other variants such as HbF, HbC and HbE. These 45 hemoglobin variants found by HPLC were confirmed using the capillary electrophoresis method.

Conclusions: In conclusion, there is a very good agreement between the different HbA1c methods, for the HbA1c measurement. Direct detection by HPLC provides a relative and accurate quantification of the hemoglobin A1c fraction. All laboratories should have other equipments with other methodologies to confirm HA1c values in the presence of hemoglobin variants and to confirm and classified the variants themselves.

P40

ATIVE B12 AND METHYMALONIC ACID IN THE ACESSEMENT OF VITAMIN B12 DEFICIENCY

Cláudia Fernandes[1], Eulália Costa[1], Cristiana Lopes[1], Fátima Amado[1], Fernando Rodrigues[1]

[1]Centro Hospitalar e Universitário de Coimbra

Introduction: Neurological and hematological disturbs can occur with vitamin B12 (B12) deficiency. This situation emerges when VitB12 is not absorb in the gastrointestinal track, including intestinal disease, pernicious anaemia and aging, or when dietary intake is not enough, as in vegan diets. B12 bonds mainly to two plasmatic proteins but the biologically available form is the one bonded to transcobalamine and represents 10-30% of the circulating B12. This form is called holotranscobalamine or active B12 (aB12) and some studies suggest that is more sensitive in the diagnosis of B12 deficiency. In cells, the lack of B12 stops some metabolic pathways and increases blood MMA and HCY.
It is often assumed that laboratory assay for serum B12 can test for the deficiency but the active form is more targeted and the functional assays, that include methylmalonic acid (MMA) and homocysteine (HCY) are important for B12 deficiency confirmation.
Sequential assays algorithms with combination of multiple markers have been proposed and can be the key to improved diagnosis.

Aim: The aim is to evaluate the assays B12, aB12, MMA and HCY in the evaluation of B12 deficiency.

Material and methods: One-year retrospective study with, at least, two of the following patient results: B12, aB12, MMA or HCY. Cutoff values were defined as: <250pg/ml for B12 (considering elderly and pediatrics); <70pmol/L (possible deficiency) and <25pmol/L (deficiency) for aB12; >0,400µmol/L for MMA and 15,0µmol/L for HCY.

Results: A total of 170 patients were selected being 156 with B12 (23 were <250pg/ml), 42 with aB12 (26 were <75pmol/L), 158 with MMA (29 were >0,400µmol/L) and 78 with HCY (19 were >15,0µmol/L). Samples with B12 <250pg/ml revealed 57,7% with MMA or HCY above cut-off and 39,1% with aB12 <75pmol/L. Samples with aB12 <75pmol/L revealed 34,6% with MMA or HCY above cut-off and all samples with aB12 <25pmol/L had MMA >0,400µmol/L. An equal number of samples (9) were found with B12 <250pg/ml and aB12 <75pmol/L and with aB12 <75pmol/L and positive functional markers for B12 deficiency.

Conclusions: the majority of samples with B12 <250pg/ml had at least one functional marker positive for B12 deficiency suggesting that additional studies have to be performed in these patients. aB12 revealed to be a good assay for clinical algorithms and to confirm vitamin B12 deficiency.

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P41

THE USE OF PROCALCITONIN DURING THE COVID-19 PANDEMIC: SINGLE-CENTRE RESTROSPECTIVE AUDIT

Lídia Carvalho[1], Miriam Gonçalves Sousa[2], João Tiago Guimarães[1]

[1]Centro Hospitalar Universitário de São João, [2]Hospital Dr. Nélio Mendonça

Procalcitonin (PCT) has been widely studied as a biomarker for early detection of infection, to predict mortality and guide antibiotic management. The COVID-19 Pandemic was a trigger to initiate PCT measuring at our hospital. Our goal was to audit the use and performance of PCT as a biomarker of infection.

PCT was measured 10 387 times in 3690 adult patients between 01-04-2020 and 31-12-2021. Patients were mostly men (62%) from the 70-79 age-group. PCT measurement was mostly used by Intensive Care Units (51%) and in COVID-19 patients (83%), with an overall mean of three determinations per patient. The mean value of PCT was 2,22ng/mL, with 30% values being above reference (>0,5ng/mL), of which 66% were from ICU patients. In order to evaluate PCT performance as a biomarker for infection we restricted our search to PCT values obtained from patients in ICU between 01-01-2021 and 31-03-2021, with C-Reactive Protein (CRP) and White Blood Count (WBC) measured from the same blood sample. A total of 1113 simultaneous determinations from 269 different patients were studied, with an overall mean of four measurements, per patient. Of these 269 patients, the mean value for PCT was 2,48ng/mL, for CRP 121,47 mg/L and for WBC 11,78 x 10^3/µL. In 25% of cases, PCT either kept up with increase or decrease in CRP and WBC, but for 36% CRP rose earlier and to a greater extent than PCT. In no case PCT was the earliest biomarker.

On 70% of our PCT measurements overall values were low (<0.5ng/mL), with higher PCT in ICU patients. Indeed, studies have shown that in the majority of the population, PCT is very low (<0.02ng/mL) for healthy individuals and low in patients with pure viral infections. Early on the COVID-19 Pandemic, some authors showed that PCT was five times higher for severe disease.

Although, for 59% of our cases, CRP and WBC combined, were more sensitive and rose earlier than PCT, this doesn´t corroborate other studies where PCT was found to be a more accurate, sensitive and precise diagnostic and prognostic biomarker.

Further studies could improve the defition of severe disease to include information about infection source, outcome for the patient and co-morbidities, in order to understand if the differences found between our conclusions and other results, could be thus explained.

P42

CHARACTERIZATION OF THE MOLECULAR PROFILE OF BEE VENOM ALLERGY

Silvia Freitas Arroja[1], Cátia Santa[1], Jorge Meneses[1], Ana Paula Cruz[1]

[1]Centro Hospitalar de Vila Nova de Gaia e Espinho, EPE

Background: Hymenoptera venom allergy (HVA) is a potentially fatal allergic condition. It is a frequent cause of anaphylaxis in adults and children and is also responsible for decreased quality of life and significant anxiety about future stings. The best characterized hymenoptera venom is that of the bee (Apis mellifera). Several major allergens such as Api m 1, Api m 2, Api m 3, Api m 5 and Api m 10 when taken together show a diagnostic sensitivity of 95%: Api m 1 (phospholipase A2) is the most frequent molecular allergen in patients with Bee Venom (BV) allergy; Api m 3 (acid phosphatase) and Api m 10 (icarapin) are also considered specific to BV allergy; Api m 2 (hyaluronidase) shares 55% sequence identity with vespid hyaluronidase and Api m 5 (dipeptidyl peptidase 4) seems to be responsible for the cross-reactivity between bees and wasps. This study aims to characterize the sensitization profile by molecular components of patients with anaphylactic reactions to BV.

Methods: Retrospective study of patients with history of systemic reactions to bee sting, including skin testing and/or sIgE antibodies with whole extracts (Apis mellifera), between July 2016 and November 2020. Serum IgE to recombinant allergens (rApi m 1, rApi m 2, rApi m 3, rApi m 5, rApi m 10) by ImmunoCAP® (Thermo Fisher Scientific®, Uppsala, Sweden). Value ≥0.35 kUA /l was considered positive.

Results: Fourteen patients were included, 78.6% male, 21.4% female, mean age 48.5 years. Serum IgE to rApi m 1 was detected in 42.9% (6/14), rApi m 2 in 21.4% (3/14), rApi m 3 in 0% (0/14), rApi m 5 in 28.6 % (4/14), rApi m 10 in 35.7% (5/14) of patients. Seven patients (50.0%) were double positive and negative results for allergens were detected in 3 patients (21.4%).

Discussion: In vitro diagnostic of HVA allows an improved differentiation of the relevant sensitization, particularly in BV allergy, because it enables us to characterize individual sensitization profiles that may be of relevance for the treatment outcome of venom immunotherapy (VIT). Increasing the number of allergens available for molecular characterization of sensitization profiles improves diagnostic accuracy and combined with the use of cross-reactive carbohydrate determinant-free recombinant allergens allows differentiation between true sensitization and cross-reactivity. Venom immunotherapy (VIT) is the only long-term curative treatment available, and Api m 10 is considered a marker of failure in BV immunotherapy.