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Congress Abstracts

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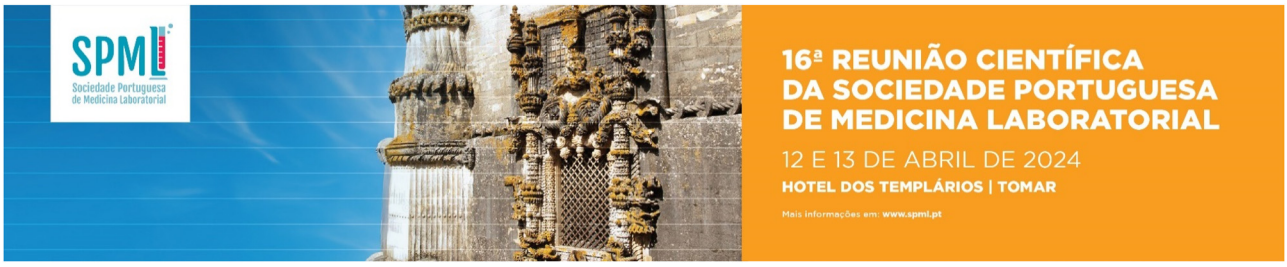

|Sessão Prémio Melhor Comunicação Oral

CO.01

KINGELLA KINGAE BACTEREMIA IN A CHILD – CASE REPORT

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Introduction: *Kingella kingae* is a facultative anaerobic, slowly growing, Gram negative coccobacillus belonging to HACEK group *(Haemophilus spp, Aggregatibacter actinomycetemcomitants, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae).* This is a commensal of oropharynx flora in healthy children aged 6 months to 4 years. Due to improved culture and identification methods, *K. kingae* is nowadays recognized more often as a pathogen in invasive infections, mainly osteo-articular, bacteremia and rarely endocarditis.

Case presentation: A 13-month-old, previously healthy female infant went to the emergency department with history of aphthous stomatitis with three days of evolution associated with fever. She also reported rhinorrhea and productive cough. Since the previous day, she showed pain and swelling on the left hand. She had no history of trauma.

On physical examination, she presented vesicular lesions and macules on the lips and perioral region and left hand with local inflammatory signs (edema and rubor), conditioning functional limitation. The rest of the examination was unremarkable.

Laboratory analysis showed a white cell count within normal range, a C reactive protein of 39.2 mg/dL and a sedimentation rate of 67 mm/1st hour. One blood culture was obtained. Plain radiograph of the affected hand was normal. Ultrasound described thickening of subcutaneous tissue, suggesting cellulitis.

Blood culture was positive after 25 hours and culture on blood and chocolate agar plate grew slowly colonies. The Gram stain showed coccobacilli Gram negative. The catalase reaction was negative and oxidase positive. Identification in matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) revealed to be *K. kingae.* Antimicrobial susceptibility test was susceptible for ceftriaxone, penicillin and trimethoprim-sulfamethoxazole. Beta-lactamase test was negative.

The child started on ceftriaxone i.v. for 2 weeks, with excellent clinical and analytical evolution. To discard valve vegetations or osteomyelitis/septic arthritis, an echocardiogram and magnetic resonance were performed and were unremarkable.

Discussion: The authors highlight the importance of identification of *K. Kingae* colonies and their fastidious nature, as essential for the high suspicion of positive cultures. The diagnosis of this blood infection must be established as soon as possible in order to start target antibiotic therapy and prevent possible complications.

CO.02

DELAYED DIAGNOSIS OF LATE-ONSET POMPE DISEASE: A CASE REPORT EMPHASIZING THE IMPORTANCE OF TIMELY CLINICAL SUSPICION AND GENETIC ANALYSIS

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Introduction: Pompe disease (MIM #232300) is an autosomal recessive disorder caused by pathogenic variants in the gene encoding lysosomal acid alpha-1,4-glucosidase (GAA), located in chromosome 17q25. The severe infantile-onset form is characterised by hypertrophic cardiomyopathy, hepatosplenomegaly, respiratory failure and asthenia. Late-onset form manifests at any age and tends to be more indolent. In both phenotypes, with decreased GAA, CK is typically elevated. Identification of pathogenic variants in GAA gene is mandatory for diagnostic confirmation. Treatment involves enzyme replacement therapies to reduce/delay the onset of symptoms.

Case Report: The index case died at the age of one with a diagnosis of Pompe. Shortly after the father, the hereby-reported case, and the carrier wife (first degree of consanguinity - second cousins) were studied. They had second child, currently an asymptomatic adult, who did a prenatal diagnosis.

The 51-year-old male patient was referred to the hospital by the family general practitioner with aggravating symptoms of weakness and asthenia, which he had experienced throughout his life. An initial enzymatic activity performed in 1997 by spectrophotometry, revealed an activity slightly below the reference range (RR). The current method uses fluorescence substrates and is more sensitive. In 2023, next generation panel approach revealed two pathogenic variants: c.-32-13T>G p.? and c.1933G>T p.(Asp645Tyr), both in heterozygosity, in the GAA gene, along with an elevated CK in the biochemical evaluation. A diagnosis of late-onset Pompe disease with significant myopathy was established, and the patient initiated enzyme replacement therapy.

Conclusion: The variant c.-32-13T>G p.? detected in intron 1 of the GAA gene is one of the most common variants in late-onset Pompe disease. The variant c.1933G>T p.(Asp645Tyr) detected in exon 14 of the GAA gene is found in trans with the pathogenic variant c.-32-13T>G p.?. In conclusion, the patient experienced symptoms of muscle weakness and asthenia throughout his life. An initial study was conducted in 1997 following the usual protocol but a definitive diagnosis of late-onset Pompe disease was only established in 2023 after symptom aggravation. Delayed diagnosis and, consequently, delayed therapy initiation lead to a decrease in quality of life. Hence, timely clinical suspicion, study, and diagnosis are crucial.

CO.03

MYCOBACTERIUM TUBERCULOSIS AND NON-TUBERCULOUS MYCOBACTERIA – DIAGNOSTIC CHALLENGES

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Tuberculosis (TB) is an infection of considerable morbidity and mortality caused by *Mycobacterium tuberculosis complex* (MTC). However, non-tuberculous Mycobacteria (NTM) have been increasingly implicated in pulmonary and extra-pulmonary disease, especially in immunocompromised individuals or patients with known pulmonary disease.

We present a case of a 37 y.o. man admitted to the Emergency Room with a history of blood-tinged sputum. CT pulmonary angiogram showed bronchiectasis and tree-in-bud pattern of probable infectious origin. In this context, microbiological study of 3 sputum samples ensued.

Given the high clinical suspicion of TB, direct PCR was performed in Sample 2, with a positive result for MTC, after which the patient initiated standard treatment for TB. Acid-fast staining (AFS) with Auramine was performed on all samples with no detection of acid-fast bacilli (AFB) in any of them. Following protocol, all samples were innoculated in MGIT broth and

incubated. Sample 3 was signaled positive at 23 days. AFS was performed with the Kinyoun technique, which showed AFB. Genotyping was performed, which identified *M. intracellulare*. Since there was a positive PCR result for MTC in Sample 2, we debated wether there could additionally be an MTC present in this sample. For this reason the culture was reincubated. A new AFS was performed after a month of reincubation, which showed 2 types of AFB – one with cord formation, typical of MTC and the other as seen before, sugestive of the already identified *M. intracellulare*. A second Genotyping was executed showing the presence of both MTC and *M. intracellulare*. After 40 days of incubation, Sample 2 also showed the 2 types of AFB, but Genotyping only detected the presence of MTC.

This case was considered interesting for several reasons: it is not usual to find MTC and NMT in the same individual and it highlights the importance of PCR as a preliminary result. It also demonstrates how the existence of reasonable doubt and critical thinking led to an action that allowed MTC detection on cultural exam, which is the gold-standard for TB diagnosis. This led to the correct treatment, which would not be possible if only the NTM was to be identified, therefore preventing further morbidity and mortality.

CO.04

CAN FLOW CYTOMETRY ANALYSIS PREDICT THE GRAM TYPE OF BACTERIA CAUSING URINARY TRACT INFECTIONS?

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Introduction: Screening of urine samples prior to urine culture on the Sysmex UF-5000 (Sysmex Corporation) automated flow cytometer allows for the swift detection and quantification of clinical parameters, including squamous epithelial cells, leukocytes, erythrocytes and bacteria (BACT), thus facilitating selection of samples requiring further laboratory processing. Currently, the analyser also has a Gram-positive (BACT:GP) / Gram-negative (BACT:GN) flag to differentiate the Gram type of BACT detected.

Aim: The aim of this study was to evaluate the accuracy of the BACT:GP/BACT:GN flow cytometer flag compared to subsequent urine culture results.

Methods: This was a one-month, single-center retrospective study of screen-positive urine samples. Flow cytometer flag info (BACT:GP, BACT:GN, BACT:GP/GN and BACT:unclassified) and urine culture results were collected and compared for each sample. Sensitivity (SN), specificity (SP), positive and negative predictive values (PPV and NPV), and Cohen's kappa (κ) were calculated.

Results: A total of 1158 screen-positive urine samples were studied. The BACT:GP flag info was correct in 47.9% (167/349) of cases, namely 41.6% (145/349) pure cultures and 6.3% (22/349) mixed cultures. SN was 83.1%, SP was 76.2%, PPV was 47.9%, NPV was 94.5%. The value of κ was 0.47 (weak agreement). On the other hand, the BACT:GN flag info was correct in 95.3% (444/466) of cases, namely 71.9% (335/466) pure cultures and 23.4% (109/466) mixed cultures. SN was 87.4%, SP was 95.2%, PPV was 95.3%, NPV was 87.2%. The value of κ was 0.82 (strong agreement). For urine samples with the BACT:GP/GN mixed flag, 53.4% (102/191) turned out to be GN BACT pure cultures, 35.6% (68/191) GN BACT mixed cultures, 5.8% (11/191) GP BACT pure cultures and 2.6% (5/191) GP

BACT mixed cultures. Regarding urine samples with the BACT:unclassified flag, 55.2% (84/152) turned out to be sterile cultures, 15.8% (24/152) GP BACT pure cultures, 11.2% (17/152) GN BACT pure cultures and 9.2% (14/152) of yeast pure cultures.

Conclusions: Our results show that the BACT:GN flow cytometer flag seems to be able to predict GN BACT causing urinary tract infections (UTI), a feature that can help guide the choice of antimicrobial therapy. Nevertheless, the BACT:GP flow cytometer flag showed a reduced PPV, probably due to our local epidemiology with low prevalence of UTI caused by this type of BACT.

CO.05

SUBCUTANEOUS INFECTION CAUSED BY ALTERNARIA ALTERNATA IN KIDNEY TRANSPLANT PATIENT

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Abstract: Fungal infections are rare in the general population, but they are an emerging cause of disease and are important factors in morbidity and mortality in immunocompromised patients and solid organ transplant patients (SOT). *Alternaria alternata* is an emerging fungus that is mainly found outdoors and normally grows on vegetation. It is a known cause of allergies and asthma but can also cause serious infections such as keratomycosis, cutaneous alternariosis, paranasal sinusitis and granulomatous pulmonary nodules. We report a case of subcutaneous alternariosis in a kidney transplant recipient.

Case report: Male patient, 59 years old, kidney transplanted since December 2022 and under immunosuppressive therapy. In October 2023, the patient suffered a pine bark injury in the anterior fascia of the left leg, initially with a blackened appearance and signs of inflammation, having been medicated for five days with amoxicillin/clavulanic acid and ciprofloxacin (250mg), without significant improvement, he went to the Emergency Department. On assessment, 4 ulcerated nodules covered in serous-hematic crust were observed on the anterior surface of the left leg, the largest measuring around 2x2 cm, with local signs of inflammation and slight pain on local palpation. Given the context of immunosuppression, a biopsy was carried out to check for opportunistic microorganisms. Although the direct examination of the tissue was negative for fungi the culture revealed the growth of dark gray colonies. Microscopic examination of the colony using lactophenol blue showed septate pigmented hyphae and chains of pyriform conidia with transverse and longitudinal septa with a short cylindrical beak. *Alternaria alternata* was identified.

Conclusion: Subcutaneous alternariosis is an opportunistic infection and the main predisposing factors for this infection are work with frequent contact with the ground, diabetes mellitus and local skin trauma associated with immunosuppression such in as recipients of solid organs. This case emphasizes that is crucial the awareness of opportunistic fungal infections and that tissue samples are the most sensitive and specific for the diagnosis. As the number of patients receiving solid organs and receiving immunosuppressive drug therapy increases, it is likely that the risk of these skin infections will also increase.

CO.06

AREA OF TECHNICAL UNCERTAINTY FOR CIPROFLOXACIN IN ENTEROBACTERALES: ONE-YEAR SINGLE-CENTER EXPERIENCE

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Introduction: An area of technical uncertainty (ATU) is a warning to laboratory staff to inform them of the equivocal interpretation of antimicrobial susceptibility testing (AST) results and thus avoid false susceptibility reports. Current ATUs are described for different bacterial genera, but are very common for ciprofloxacin (non-meningitis) in *Enterobacterales*. Since there is no mandatory action to an ATU, the warning can be ignored or acted upon.

Aim: The aim of this study was to assess the ability to overcome the ATU for ciprofloxacin in *Enterobacterales* through an alternative test based on the determination of the minimum inhibitory concentration (MIC).

Methods: This was a one-year, single-center retrospective study based on a convenience sampling of clinical isolates of *Enterobacterales*, including *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), during January to December 2023. Initial AST was carried out using the VITEK® 2 (bioMérieux) analyzer. In the presence of the automatic ATU for

ciprofloxacin flag, the determination of the MIC was performed using gradient strip diffusion, and results were interpreted according to EUCAST 2023 clinical breakpoints, that is susceptible (S) ≤ 0.25 mg/L, resistant (R) > 0.5 mg/L and ATU = 0.5 mg/L.

Results: A total of 33 clinical isolates of *Enterobacterales* were included. The determination of the MIC was able to surpass the ATU for ciprofloxacin in 63.6% (n=21/33) of cases, allowing their recategorization as S in 90.5% (n=19/21) and as R in 9.5% (n=2/21). However, the MIC value of the remaining 36.4% (n=12/33) of cases persisted within the ATU range, so it was best decided to downgrade the susceptibility categorization (I → R).

Conclusions: Our results show that the determination of the MIC is a good strategy to overcome the ATU for ciprofloxacin in *Enterobacterales*. Surprisingly, this alternative allowed recategorization as S in most cases, which restores the ability of the clinician to use this antimicrobial agent whenever necessary, and does not overestimate local epidemiological data.

CO.07

SIMULTANEOUS ACUTE SPLENIC SEQUESTRATION AND TRANSIENT APLASTIC CRISIS IN A PATIENT WITH HBSC

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[1]*ULSGE*

Introduction: In individuals with the SC genotype each parent contributes one copy of hemoglobin S and one copy of hemoglobin C. Consequently, the composition of hemoglobin in HbSC erythrocytes is approximately 50% HbS and 50% HbC. The clinical abnormalities in Hemoglobin SC disease arise from the interaction between HbS and HbC. HbC enhances the formation of intracellular polymer of HbS by dehydrating red blood cells.

Case Report: A 33-year-old patient with Hb SC disease, presented to the emergency department with severe pain radiating to the lower limbs. Physical examination revealed splenomegaly. Laboratory values on admission were hemoglobin (Hb) 8.6 g/dL, hematocrit 22.8%, white cell count 8.3 × 10^6/L, platelets 241 × 10^6/L, and MCV 69.5 fL with frequent target cells and drepanocytes. Haptoglobin levels <10 mg/dL, LDH 367 U/L, troponin 1.9 ng/L, and total bilirubin 0.73 mg/dL. The hemoglobin high-performance liquid chromatography showed a pattern compatible with SC

Hemoglobinopathy, with 43.5% of HbS, 36.5% of HbC and 1.3% of HbF. Uncontrolled pain, the development of acute-onset dyspnea and oxygen desaturations correlating with worsening laboratory parameters led to the patient being transferred to an intensive care unit with acute coronary syndrome requiring mechanical ventilation. Laboratory values were now Hb 4.3 g/dL, WBC 12.24 × 10^6/L and platelets 78 × 10^6/L with polychromasia and numerous erythroblasts. LDH 2006 U/L, troponin 85 ng/L, NT Pro-BNP 1337 pg/mL, CRP 22.53 mg/dL and procalcitonin 0.09 ng/L. The diagnostic hypothesis included splenic sequestration, considering splenomegaly and a considerable drop in Hb and thrombocytopenia. However, reticulocytopenia since admission and good transfusion profitability suggested probable transient red cell aplasia (TRCA) that was demonstrated by the presence of immunoglobulin M of parvovirus B19.

Discussion: TRCA is due to human parvovirus B19 infection and results in severe anemia, accompanied by reticulocytopenia. Acute splenic sequestration crisis (ASSC) is characterized by increasing splenomegaly and a sudden fall of 2 g/dL in Hb concentration. In contrast to TRCA, acute anemia is accompanied by reticulocytosis, circulating nucleated red blood cells and thrombocytopenia due to the trapping of red cells and platelets in the spleen. Both these complications have presentation differences, being mutually exclusive. However, there have been few cases reported where ASSC occurred in association with TRCA.

CO.08

AREA OF TECHNICAL UNCERTAINTY FOR PIPERACILLIN/TAZOBACTAM IN ENTEROBACTERALES: ONE-YEAR SINGLE-CENTER EXPERIENCE

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Introduction: An area of technical uncertainty (ATU) is a warning to laboratory staff to inform them of the equivocal interpretation of antimicrobial susceptibility testing (AST) results and thus avoid false susceptibility reports. Current ATUs are described for different bacterial genera, but are very common for β-lactam/β-lactamase inhibitors, including piperacillin/tazobactam (P/T), in *Enterobacterales*. Since there is no mandatory action to an ATU, the warning can be ignored or acted upon.

Aim: The aim of this study was to assess the ability to overcome the ATU for P/T in *Enterobacterales* through an alternative test based on the determination of the minimum inhibitory concentration (MIC).

Methods: This was a one-year, single-center retrospective study based on a convenience sampling of clinical isolates of *Enterobacterales*, including *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), during January to December 2023. Initial AST was carried out using the VITEK® 2 (bioMérieux) analyzer. In the presence of the automatic ATU for P/T flag, the determination of the MIC was performed using gradient strip diffusion, and results were interpreted according to EUCAST 2023 clinical breakpoints, that is susceptible (S) ≤ 8 mg/L, resistant (R) > 8 mg/L and ATU = 16 mg/L.

Results: A total of 76 clinical isolates of *Enterobacterales* were included. The determination of the MIC was able to surpass the ATU for P/T in 71.1% (n=54/76) of cases, allowing their recategorization as S in 77.8% (n=42/54) and as R in 22.2% (n=12/54). However, the MIC value of the remaining 28.9% (n=12/76) of cases persisted within the ATU range, so it was best decided to downgrade the susceptibility categorization (I → R).

Conclusions: Our results show that the determination of the MIC is a good strategy to overcome the ATU for P/T in *Enterobacterales*. Surprisingly, this alternative allowed recategorization as S in most cases, which restores the ability of the clinician to use this antimicrobial agent whenever necessary, and does not overestimate local epidemiological data.

CO.09

CASE SERIES REPORT: HEPARIN-INDUCED THROMBOCYTOPENIA

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Heparin-induced thrombocytopenia (HIT) is a potentially lethal, immunologically mediated adverse drug reaction to unfractionated heparin or, less commonly, to low-molecular weight heparin.

In this study, the correlation between frequently used pre-test scores and laboratory results supporting the diagnosis of HIT was evaluated.

An observational retrospective study was conducted in which patients who tested positive in the year 2023 using the STic Expert HIT test were selected (n=7). This test allows for the qualitative detection of IgG antibodies against PF4/polyanion complexes in citrated plasma or human serum, with 100% sensitivity and 93% specificity. Subsequently, based on the patients' clinical records, the pre-test probability according to the HIT 4T Score and HIT Expert Probability (HEP) Score was studied. The patients were diagnosed with HIT, on average, on the 4th day after exposure; none of them had been exposed to heparin in the previous 6 months, 71% were exposed to unfractionated heparin, 86% switched to argatroban after diagnosis was established, having recorded a mortality rate of 43%. The Wilcoxon test was used to compare the STic Expert HIT results with those obtained through the HIT 4T Score and HEP Score to understand which score correlates better with the diagnosis.

Current laboratory diagnostic tools incur significant time delays before confirming HIT, hence the importance of pre-test evaluation based on established scores, as treatment involves immediately ceasing the use of heparin. In this study, a statistically significant difference (w= 2.5, p-value= 0.0469) between the two scores analyzed was obtained, concluding that the HEP Score has a greater concordance with the confirmed clinical diagnosis.

In accordance with the literature, the HEP Score has shown significantly greater interobserver agreement, correlation with results of HIT laboratory testing, and concordance with the diagnosis of an expert panel. It has been reported to have a high sensitivity and a moderate specificity for determining the presence of HIT as defined by an expert panel, which could potentially reduce the use of direct thrombin inhibitors by 41%. Meanwhile, the HIT 4T Score, which has been widely used in clinical practice, also has its merits, and its predictive value for HIT has been validated in diverse settings and patient populations. However, when directly compared, the HEP Score appears to outperform the HIT 4T Score in terms of its operating characteristics.

Table 1: Population characteristics. [1]from patient admission to the date the STic Expert HIT test was requested

Total of patients, n=7	**n (%)**
Age, years (median)	68
<65 years	2 (28%)
≥65 years	5 (72%)
Gender	
Male	4 (57%)
Female	3 (43%)
Platelet Count (median)	
On admission	191x10^3/µL
On the date of STic Expert HIT test	60 x10^3/µL
Nadir	460x10^3/µL
72h after stopping heparin	75.5x10^3/µL
% Platelet drop[1]	68%
Thrombotic Events	1 (14%)
Positivity for PF4 antibodies	7 (100%)
Average number of days of exposure to heparin	4

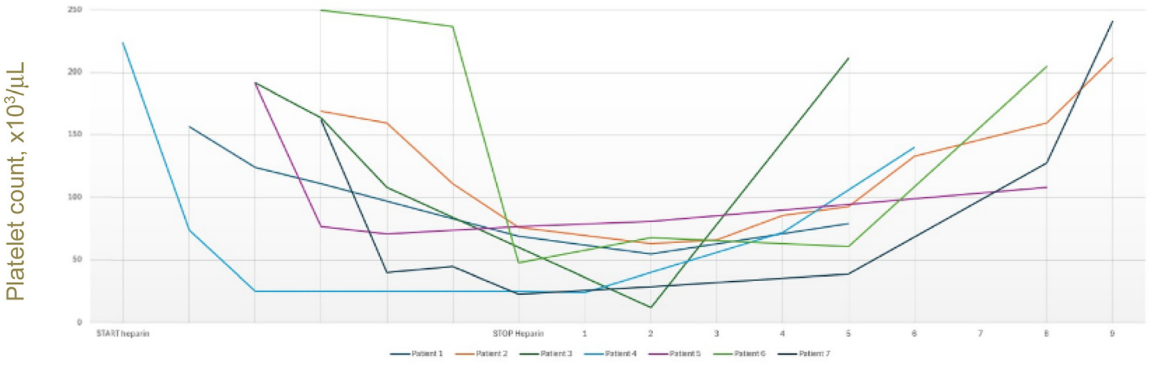

Image 1: Daily timeline of platelet count evolution by patient.

CO.10

ASSESSING PLACENTAL XENOESTROGENIC LOAD: THE LINK BETWEEN PESTICIDE EXPOSURE, MATERNAL PROFILE AND ESTROGENIC EFFECTS

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Introduction: Endocrine-disrupting chemicals (EDCs), including organochlorine compounds (OCs) such as pesticides, can disrupt hormonal balance and adversely affect health. There is limited evidence on their impact during pregnancy, a critical period for foetal development. In this context, this study aims at exploring the link between the maternal profile and the levels of OCs in the placenta, along with their estrogenic effects.

Materials and Methods: A cross-sectional study was conducted in a hospital setting, where 41 placental samples were collected from healthy pregnant women. Information on sociodemographic aspects, reproductive history, consumption habits, and lifestyle was obtained via a structured questionnaire. The placental samples were assessed for the content of 14 different OCs using Gas Chromatography-Mass Spectrometry (GC-MS). Estrogenic activity was evaluated through the E-Screen bioassay. A liquid chromatography method was developed to separate xenoestrogens from natural oestrogens, categorizing them into lipophilic xenoestrogens (α fraction) and endogenous hormones (β fraction).

Data analysis comprised multivariate statistical analysis using MULTBIPLOT, including hierarchical clustering with Euclidean distance, HJ-BIPLOT scores, and Ward's method for linkage.

Results: Our findings reveal the presence of various pesticides with estrogenic activity in the placentas of women from Southern Portugal. Estrogenic activity, measured in the α-fraction, was detectable in 71% of samples. Multivariate analysis identified three distinct clusters with distinct maternal profiles. Urban women, particularly those consuming a high-fat diet, exhibited a higher number and concentration of analytes, along with increased estrogenic activity in the α fraction. These women were predominantly multiparous, had a higher body mass index before pregnancy, and experienced more significant weight gain during pregnancy, leading to the birth of heavier babies.

Conclusion: This study highlights the significant impact of maternal dietary and lifestyle factors on placental accumulation of organochlorine pesticides and their estrogenic effects, while encouraging follow-up studies targeting the potential adverse effects on foetal development.

CO.11

RETICULOCYTE HEMOGLOBIN CONTENT: IS IT VALUABLE BEFORE SCHEDULED ORTHOPEDIC SURGERY?

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Introduction: Patients undergoing surgery are typically evaluated with pre-procedure blood work to screen for conditions that may predispose them to complications during or after the procedure. Studies have indicated that reticulocyte hemoglobin content (Ret-He) is associated with higher transfusion requirements in critically ill patients. The authors hypothesize whether this parameter holds the same utility in scheduled orthopedic surgery. The objective of the present study is to assess if there is an association between Ret-He and a higher transfusion requirement in patients undergoing scheduled orthopedic surgeries.

Methods: A retrospective cohort study was conducted on patients undergoing scheduled orthopedic surgery in a Portuguese hospital. Collected data included age, gender, presence of anemia or medication directed at this condition, previous transfusion need, isoimmunization, and heart failure, as well as analytical values of complete blood count with reticulocytes, biochemistry, and coagulation study. The cut-off for classifying Ret-He as low was set at < 29 pg.

Results: This study included 113 patients, with a median age 68 years-old [IQR 59-75]. Median Ret-He was 34.3 [IQR 33.2-35.88] pg, and only 3 patients had low Ret-He < 29 pg. None of these patients received a blood transfusion until discharge. Twelve patients required transfusion (10,6%), and all of them had Ret-He >29 pg. Additionally, between blood transfusion recipients (34.4 pg [IQR 32.7 – 36.8]) and the remaining patients (34.2 pg [IQR 33.1 – 35.7]), no statistically significant differences were found in Ret-He (p=0.6). The individual cost of this parameter was calculated as 3.95€ in this laboratory, and the annual cost for this population of patients undergoing scheduled orthopedic surgery was 3,598€.

Conclusion: In the studied population, Ret-He did not prove to be a useful discriminator of transfusion needs and might not be useful in this clinical context. As this analysis represents an additional cost of care, it is important to reflect about the usefulness of the parameter.

CO.12

PROCALCITONIN AND NEONATAL SEPSIS: IS IT ACCEPTABLE TO JUMP BETWEEN SERUM AND EDTA-3K PLASMA?

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Introduction: Procalcitonin (PCT) concentration increases during bacterial infections, being useful for the diagnosis of neonatal sepsis. In our laboratory PCT is measured in serum and technical specifications mention that we should use the same matrix for patient monitorization. However, since neonates' samples arriving at the laboratory are usually insufficient, occasionally one may need to use a different tube to optimize the volume of sample available for the analytical study.

Objectives: Evaluate the difference in PCT concentration in serum and EDTA-3K plasma for possible use of plasma when serum is scarce, particularly in neonates.

Material and methods: We measured PCT in 30 samples in an EDTA-3K tube when PCT value in serum had been previously measured. The method was Abbott's *ALINITY* i B·R·A·H·M·S *PCT, a* chemiluminescent microparticle immunoassay, in Alinity ci-series. Both samples were collected at the same time and the EDTA was stored for up to 8 hours at room temperature before centrifugation and processing in accordance with the technical specifications provided by Abbott®. The statistical analysis was performed using MedCalc® Statistical Software version 22.013 with simple linear regression, Pearson correlation and Bland–Altman plot.

Results: Correlation coefficient (R2) was >0,99 meaning that there is a very high correlation between the variables. Linear regression shows a correlation with a slope of 1,0916, indicating positive correlation as serum concentration increases. PCT average measurement was higher in EDTA-3K plasma compared to serum. Bland-Altman plot revealed a growing difference as serum PCT increases, with an average difference of -12,1% when plotted against Plasma.

Conclusion: There is no significant difference in lower concentrations between the two matrices (in absolute value) around the threshold considered for the diagnosis of neonatal sepsis. EDTA-3K could be used to replace serum for PCT measurements in neonates when there is scarcity of blood sample and a diagnostic result must be produced. However, for monitorization purposes, when there are multiple matrices at use, the clinician should be informed of which sample was processed and the possible variation in the result, providing for a correct interpretation of PCT value according to clinical relevance and context.

CO.13

INTRAOPERATIVE PARATHYROID HORMONE: HERO WITHOUT A CAPE! CLINICAL CASE

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Introduction: Parathyroid hormone (PTH) measured during parathyroidectomy has an unequivocal contribution to surgical success since more than one gland can be affected and find outside their usual location.

The availability of a rapid test, combined with PTH short half-life (5 min), allows for serial intraoperative PTH measurements (ioPTH) in the lab. The complete removal of hypersecretory tissue - adenoma, carcinoma or hyperplasia - results in >50% drop in PTH level every ±5 min. It should be observed, at least 2 consecutive falls in PTH baseline level to confirm the absence of remaining hypersecretory tissue and consequent removal of the affected gland(s).

Case report: A 75-year-old woman with primary hyperparathyroidism (PHPT); imaging tests revealed: 1 - parathyroid adenoma posterior to the lower pole of the thyroid right lobe; 2 - thyroid right lobe nodule with a follicular lesion of undetermined significance; 3 - inferior right side paratracheal lesion described as a bronchogenic cyst. She was proposed to right inferior parathyroidectomy.

During surgery, the right inferior parathyroid (#1) was excised but an increase in ioPTH serum levels was observed. This result led to exploration of the right parathyroid surgical field and right hemithyroidectomy (#2) but ioPTH levels remained unchanged. In the absence of ioPTH levels decrease, the paratracheal lesion described as a bronchogenic cyst was excised (#3). The observed decrease in ioPTH levels led to the conclusion that the probable cyst was, in fact, the hypersecretory parathyroid gland. Extemporaneous examination of the removed tissue was suggestive of parathyroid tissue.

Histological results revealed: #1 - normal parathyroid tissue; #2 - nodular follicular thyroid disease; #3 - cystic parathyroid adenoma.

Conclusion: The surgeon's expertise, allied with preoperative localization techniques and laboratory's ioPTH monitoring, is crucial during the surgery and is a determining factor of PHPT cure. It allows for minimally invasive surgery, resulting in reduced hospitalization time and less patient morbidity.

In more complex cases, as the one presented, in which initial surgical proposal did not prove to be the right solution, ioPTH assay helped the surgeon to find the affected gland, resulting in patient's cure and avoiding further surgery.

CO.14

WHAT HAPPENS IN GATA2 GENE MUTATION?

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Introduction: The GATA2 deficiency syndrome (G2DS) is a rare autosomal dominant genetic disease caused by germline heterozygous variants of this gene. The prevalence is currently unknown. This condition predisposes individuals to a spectrum of diseases. The revised 2016 WHO classification introduced a novel category of "myeloid neoplasms with germline predisposition" with *GATA2* and other genes, expanding the spectrum of hereditary myeloid neoplasms. The deficiency is diagnosed based on clinical findings, laboratory, and genetic testing. The disease may initially manifest as myelodysplastic syndrome, as seen in 75% of cases, such as the patient we are presenting.

Clinical Case: An 11-year-old girl was referred to Hematology consultation due to multiple warts, macrocytosis, and mild neutropenia. Full blood count showed: Erythrocytes 3.44x10^12/L, Hemoglobin 12.0x10g/L, MCV 98.5fL, Leucocytes 4.13x10^9/L, Neutrophils 0.61x10^9/L, Eosinophils 0.04x10^9/L and Platelets 236x10^9/L. Chemical analysis demonstrated normal vitamin

B12 levels and folic acid deficit. Bone marrow aspirate revealed: decreased cellularity with hypoplastic granulomonocytic series; increased erythroid series with macrocytosis and megakaryocytic series with small cells and dysmorphic segmentation; hypoplasia of the granulomonocytic series; hyperplasia of the erythroid series with macrocytosis and megakaryocytic series with small megakaryocytes and dysmorphic segmentation. Histological study also proposed the hypothesis of myelodysplasia. The genetic study identified variant *c.1017+572C>T* in intron 4 of the *GATA2* gene in heterozygosity, classified as pathogenic. Immunophenotyping of peripheral blood revealed absolute and relative B and NK lymphopenia and cytogenetic study monosomy of chromosome 7.

Patient underwent stem cell transplantation and is under observation by a multidisciplinary team.

Conclusion: Germline variants in *GATA2* are associated with deficiency syndrome, with predisposition to myelodysplastic syndrome, acute myeloid leukemia and blast transformation of chronic myeloid leukemia.

The aim of studying this pathology is to deepen our understanding of the biology of hematopoietic stem cells, thereby contributing to advancements in disease treatment and providing genetic counseling for patients.

CO.15

THE UTILITY OF FIB-4 INDEX AS A TOOL TO PREDICT LIVER FIBROSIS IN PRE-DIABETES

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Introduction: Metabolic dysfunction-associated liver disease (MASLD) affects 32% of adults, is a common cause of chronic liver disease, and leads to liver fibrosis, cirrhosis, hepatocellular carcinoma, and eventually liver transplantation (1). Notably, in metabolic risk groups, it exceeds 70% in type 2 diabetes patients (DM) and reaches 90% in obese individuals requiring bariatric surgery (2). Most patients are asymptomatic, identified only through liver enzymes in routine tests. The FIB-4 index requires age, platelet count, and liver enzymes (AST, ALT), and has a high negative predictive value (NPV) for low-risk fibrosis (FIB-4<1.30), aiding accurate risk stratification and referral of high-risk patients to specialists (FIB-4>2.67) (3).

Aim: To evaluate, based on FIB-4, the degree of liver fibrosis in pre-diabetic (preDM) and diabetic patients (DM) with steatohepatitis.

Methods: A cohort of outpatients aged 35-65 years, monitored at a laboratory, with elevated AST/ALT were retrospectively analyzed (2021-2023) and stratified according to their fasting blood glucose into preDM (>110<126 mg/dL) and DM (≥126 mg/dL). Patients who presented positive antigens or antibodies to Hepatitis B/C viruses were excluded. FIB-4 assessed liver fibrosis risk using anonymized electronic data. Alcohol abuse was assessed indirectly by AST/ALT ratio (>1.5).

Results: In a total of 647 patients, 54.7±7.3 years old, 75% were male. PreDM and DM were present in equal percentage. According to FIB-4 index, 35,5% of patients had NPV for low-risk fibrosis (FIB-4<1.3). Among those 64,5% with FIB-4 >1.3, 11% had alcohol comorbidity. When stratifying by blood glucose level, 63% and 65% had FIB-4>1.3 depending on if they were preDM or DM, respectively, but those requiring referral to specialist was higher in preDM (17%) compared to DM (14%).

High-risk fibrosis results exceeded those in European and American literature (4–6).

Conclusions: FIB-4>1.3 suggest higher risk of advanced liver fibrosis and the need for monitoring and further evaluation. Limitations included absence of hepatotoxic medication and body mass index data. Despite no significant FIB-4 differences between preDM and DM, this study sheds light to the relevance of providing FIB-4 index estimation not only for those diabetic but also for those at diabetes risk.

CO.16

BEYOND THE NUMBERS: STATISTICAL EXAMINATION OF THALASSEMIA PATIENTS RESULTS IN A TERTIARY REFERRAL CENTER - HOSPITAL SANTA MARIA, LISBON

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Thalassemias are inherited disorders disrupting globin chain synthesis, with α-thalassemia and β-thalassemia being associated with deletions and mutations. Diagnosis involves complete blood counts to detect microcytosis and hypochromia, hemoglobin electrophoresis, and high-performance liquid chromatography to detect and classify thalassemias. Molecular genetic testing identifies specific mutations or deletions, aiding in assessing severity and treatment strategies. The most widespread deletion in α-thalassemia globally is the 3.7kb deletion, while the most frequent mutation in β-thalassemia within the Mediterranean region is at codon 39.

This observational study aims to characterize the age- and sex-predominance and the prevailing deletions/mutations in thalassemias of a specific population in a tertiary referral center, from patient records collected within a 5-year period (2019-2023).

LabVienna tests - Reverse hybridization StripAssay method (Kits: α-Globin StripAssay ® - covering 21 mutations; ß-Globin StripAssay ® MED, IME, SEA - each covering 22 mutations). Inclusion criteria: microcytosis (< 80fl) and hypochromia (< 27pg) with or without anemia followed by a full iron panel. For ß-thalassemia it is required that HbA2 is increased (> 3,5%) to proceed to genetic testing of ß-thalassemia.

Over this 5-year period, 852 α-thalassemia and 531 ß-thalassemia tests were requested, but only 286 (33.6%) and 132 (24.9%) met the inclusion criteria, respectively. The patient sample showed a higher female prevalence in both conditions, with ratios of 1.6:1 for α-thalassemia and 1.2:1 for ß-thalassemia. The median ages were 28 for α-thalassemia, with the highest diagnosis rates in children aged 1-10 years, and 46.5 for ß-thalassemia, most frequently diagnosed in adults aged 31-40 years. The 3.7kb deletion was the most common in α-thalassemia, with 158 heterozygous and 122 homozygous cases. For ß-thalassemia, the most common mutations were heterozygous codon 39 [C>T] and IVS 1.1 [G>A], found in 43 and 34 cases, respectively, from tests using the MED kit. At this tertiary center in the Mediterranean area, the pattern of α-thalassemia deletions matched global trends, mirroring the situation with β-thalassemia patients, who also displayed mutations consistent with those typically found in this region.

CO.17

INFECTED INTRAMUSCULAR HAEMATOMA: A RARE COMPLICATION OF INVASIVE PNEUMOCOCCAL DISEASE

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Introduction: *Streptococcus pneumoniae* is a leading cause of bacterial pneumonia and meningitis, and it is still one of the major causes of morbidity and mortality worldwide. In certain situations, for example after trauma, colonizing strains, particularly if encapsulated, can gain access to normally sterile body sites and thus cause invasive pneumococcal disease (IPD). We describe a rare case of infected intramuscular haematoma caused by a highly mucoid strain of *S. pneumoniae* in a critically ill patient.

Case Description: A 64-year-old indigent, smoker and alcoholic male patient presented to the emergency department after a fall from standing height resulting in trauma and haematoma to the right lower limb, and was discharged with symptomatic treatment. Five days later, he was admitted to the intensive care unit due to respiratory failure and severe hyponatraemia. Chest X-ray showed a left pulmonary consolidation consistent with community-acquired pneumonia, and pneumococcal urinary antigen test was positive. Moreover, lower limb CT scan showed two abscesses adjacent to the right greater

trochanter and ipsilateral thigh, so a haemato-purulent discharge sample was drawn and sent for microbiology. Gram staining revealed numerous polymorphonuclear cells and Gram-positive diplococci. After 24 hours of incubation at 35±2°C in CO_2, culture on blood agar demonstrated α-haemolytic and highly mucoid colonies, which were subsequently identified through the optochin susceptibility test and the bile solubility test as *S. pneumoniae*. Antimicrobial susceptibility testing (AST) using the Kirby-Bauer method and interpreted according to EUCAST criteria revealed a multi-drug susceptible strain. Blood cultures remained negative.

Discussion: IPD is clinically indistinguishable from other bacterial infections. However, when isolated from normally sterile body sites, *S. pneumoniae* should always be considered a true pathogen. Bacteraemia leading to cellulitis has been reported as a complication of pneumonia, mostly occurring in patients who have underlying risk factors including, as seen in this case, homelessness and alcohol use disorder. Although blood cultures were negative, the most plausible explanation for this rare form of IPD was hematogenous spread from the lower respiratory tract

CO.18

HELICOBACTER PULLORUM AN EMERGING ZOONOTIC PATHOGEN

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Introduction: Zoonotic pathogens like Helicobacter pullorum (H. pullorum) affect human and animal health. H. pullorum, identified in 1994, colonizes the digestive tract of poultry and other animals. It's transmitted through contaminated meat and can cause human illness. Diagnosing H. pullorum is challenging due to demanding culture conditions and potential misidentification with other enteric pathogens.

Clinical Case: An 84-year-old female patient, seen at a gastroenterology outpatient clinic for Crohn's disease with a 30-year history, was medicated with mesalazine 3g/day. She began with a clinical picture of alternating diarrhea and constipation, 2-3 bowel movements a day, occasional hematochezia, abdominal pain in the right quadrant, and a weight loss of 3 kg in one week. A stool sample was sent to the microbiology laboratory for bacteriological study, which was cultured on selective Campylosel agar medium and incubated in a microaerophilic atmosphere at a temperature of 42ºC. After 48 hours of incubation, small, transparent, and shiny colonies were observed, positive for the oxidase test, similar to bacteria belonging to the Campylobacter genus. Gram staining of the colonies showed the presence of small, curved Gram-negative bacilli. Helicobacter pullorum was then identified using a Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The sample was also cultured on media for the detection of other common diarrhea-producing pathogens, such as Salmonella, E. Coli O157, Shigella, and Yersinia, all of which were negative.

Conclusion: H. pullorum, an emerging zoonotic microorganism, can cause severe diarrhea and cholestasis. Although it has already been isolated in healthy individuals, in people with compromised intestinal integrity, as in the clinical case described, the consumption of meat contaminated with this pathogen can represent a risk of acute gastroenteritis or a clinical worsening of intestinal disease.

The microbiological diagnosis of H. pullorum can be challenging, and best practices are crucial for diagnosing this agent. These include adhering to the specifications of the culture media, maintaining a microaerophilic environment with an incubation temperature of 42ºC, and identifying suspicious colonies using MALDI-TOF MS.

CO.19

NO PERITONEAL FLUID SAMPLE AFTER ALL!

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Introduction: Several automated haematology analysers offer body fluid modes to evaluate cellular composition. As with any automated methodology acceptability criteria should be carefully defined according to the equipment's performance. Anticipating possible interferences that may be present in each type of sample is equally important. This case reminds us that automated analysis alone can lead to the loss of crucial information for the characterization of peritoneal fluid (PF) samples.

Case Report: A 70-year-old female underwent total colectomy and terminal Brooke ileostomy due to an obstructing descending colon neoplasm. Eleven days after surgery, loculated ascites with imaging features suggesting peritonitis required the placement of a pigtail catheter in the peritoneal cavity. The following day, a brownish PF sample was received in the laboratory for cellular composition evaluation. Analysis on the Body Fluid mode of Sysmex XN-10 revealed red blood cells 2100/µL and white blood cells 871/µL (658 polymorphonuclear and 213 mononuclear). No flags were activated. On scattergram observation cell clusters were poorly defined suggesting debris presence. Hence, microscopic observation ensued revealing numerous elements of faecal origin. Chemical analysis showed glucose < 2 mg/dL, total proteins 0.39 g/dL, albumin 0.1 g/dL, lactate dehydrogenase 6U/L and total amylase < 3 U/L. Total bilirubin was added afterwards (15.1 mg/dL).

Antibiotherapy was switched to imipenem and linesolide. Five days later, contrast fistulography confirmed the location of the drain tip within the distal ileum loop after an inconclusive attempt by CT scan. The drain was removed and replaced in the loculated ascites region. The initial drain was presumed to have migrated due to a likely connection between the ileal loop and the loculated ascites.

Discussion: Complications associated with colorectal surgery involving resections and anastomoses are not uncommon and often serious. This case highlights that PF of abnormal colour should raise suspicion and automated analysis scattergram inspection is mandatory to assess cell cluster distribution, even in the absence of flags. Microscopic observation was crucial in determining the composition of a supposed PF sample thus pointing to its intestinal origin.

CO.20

IS THERE A PLACE FOR MANUAL URINE MICROSCOPY IN AN AUTOMATION ERA? AN ELUCIDATIVE CASE OF ACUTE TUBULAR NECROSIS

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Introduction: In recent years automated urinalysis has had important developments. Its use is imperative given the high number of requests. Despite its advantages not all urinary particles are automatically identified and algorithms for result validation are lacking. Identifying renal tubular epithelial cells (RTEC) is challenging for inexperienced analysts even by light microscopy. Some commercial equipments do not automatically identify RTEC while images generated by others do not allow their identification. Acute tubular necrosis (ATN) biomarkers are limited and not widely used routinely thus, RTEC remain a hallmark of ATN diagnosis. This case exemplifies the importance of a carefully defined validation process in order to detect RTEC and possible ATN.

Case Report: A request for urinalysis was received from a 4-month-old boy admitted in an intensive care unit due to necrotizing pneumonia and bacteraemia by Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* following metapneumovirus bronchiolitis. He was medicated with salbutamol, ipratropium bromide, methylprednisolone, clindamycin and flucloxacillin, after 3 days of ceftriaxone. Diuresis and renal function were preserved. Automated urinalysis was performed in a Sysmex modular system (UC3500, UF-5000 and UD10). The urine was clear, light yellow, with pH 7.0, density 1.015 and trace haemoglobin. Flow cytometry showed leukocytes 75/µL, hyaline casts 3.28/µL, pathological casts 2.28/µL and RTEC 101/µL. Further confirmation was required due to the discrepancy between leucocyte count and absent esterase reaction, pathological casts and RTEC above our acceptability threshold. Given the limited photographic quality of automated microscopy, classification of casts and RTEC was performed using phase contrast microscopy and a stained cytospin smear. This procedure confirmed the presence of numerous RTEC and RTEC casts compatible with ATN.

Discussion: The equipment and algorithm steps taken were effective in RTEC detection and in suggesting a likely diagnosis of ATN. The ability to identify urinary RTEC and imaging may vary among analysers. Also, automated urinalysis must follow robust testing algorithms defined by experienced laboratory professionals.

CO.21

DECIPHERING MYELODYSPLASTIC SYNDROME AFTER BONE MARROW TRANSPLANTATION: ORIGINS IN DONOR OR RECIPIENT CELLS?

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Introduction: Donor cell myelodysplastic syndrome (donor-cell MDS) represents an hematologic malignancy that arises in donor cells following allogeneic haematopoietic stem cell transplantation (alloHSCT).

In patients previously transplanted for acute myeloid leukaemia (LMA)/myelodysplastic syndrome (MDS), relapse of the original disease is far more likely than MDS arising in donor cells. In contrast, in patients transplanted for other conditions, MDS is more likely to be of donor origin.

Proposed mechanisms leading to donor-cell MDS include: occult leukaemia in the donor, bone marrow microenvironment, genetic susceptibility, immune-mediated phenomenon, infection, transmission of an oncogene from the host's original disease into donor cells, toxicity of post-transplant therapies, and possibly graft versus host disease.

Case report: Fifteen-year-old male patient, diagnosed with severe aplastic anaemia in July 2015, eventually secondary to acute autoimmune hepatitis that had occurred 3 months before the diagnosis.

He was treated with alloHSCT from an unrelated donor in November 2015.

In 2020, he experienced a period of anaemia that responded to immunoglobulins and corticosteroids, at which time bone marrow was evaluated with no evidence of disease but already showing dysmyelopoiesis.

Approximately 3 years later, he developed anemia that did not respond to instituted therapy. Investigation revealed myelodysplastic syndrome with 6.3% blast cells in bone marrow evaluation with complete chimerism.

Evaluated in a bone marrow transplant service group consultation, the patient was recommended for a new allogeneic transplant.

Discussion: The development of a novel haematologic malignancy after alloHSCT is rare. So, the detection of donor-cell MDS requires a high index of suspicion.

In this reported case, the development of anaemia and dysplastic changes in the myeloid lineage morphology were important clues to the diagnosis of MDS in the transplanted patient. In this case, is more likely to be of donor origin, since there was an 100% donor engraftment during the whole post-transplant period.

In the setting of post-transplant MDS, it is important to determine the origin of the disease (recipient versus donor origin) as this has implications for treatment.

CO.22

EIKENELLA CORRODENS IN A PATIENT WITH TUBO-OVARIAN ABSCESS

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Introduction: Eikenella corrodens is a microorganism of the HACEK group, a slow-growing facultative anaerobic gram-negative rod that is part of the human oropharyngeal and genitourinary microbiome. However, despite being a commensal

of the human body, when translocated to other anatomical sites it can cause serious infections. The authors describe a pelvic inflammatory disease complicated by a tubo-ovarian abscess, with isolation of E. corrodens.

Clinical case: A 49-year-old female patient, who has been using the copper intrauterine device (IUD) as a contraceptive method for 2 years, went to the Emergency Service due to fever, abdominal pain with 4 days of evolution in the left iliac fossa (FIE), resistant to therapy with analgesia and associated with constipation. On gynecological physical examination, she presented defense to palpation of the FIE, presence of abundant vaginal discharge, yellowish and with a fetid smell. In the trans abdominal ultrasound (TA) with a heterogeneous image, suggestive of left tubo-ovarian abscess with 80x65x61mm. Analytically, she presented with leukocytosis and neutrophilia, severe anemia and elevated inflammatory parameters. She started empirical antibiotic therapy with piperacillin/tazobactam every 8 hours. She underwent exploratory laparotomy with total abdominal hysterectomy with bilateral adnexectomy. From the sample taken intraoperatively from the abscess, a microbiological identification study was carried out, identifying Eikenella corrodens by MALDI-TOF mass spectrometry and in the antimicrobial susceptibility test, it was multisensitive.

Conclusion: E. corrodens, one of the main agents of periodontal disease, also plays a significant role in other severe infections, including opportunistic infections. This microorganism has a tendency to cause abscesses in various locations. Although rare, cases of chorioamnionitis or tubo-ovarian abscesses have been reported in women who use IUDs. An important microbiological point to highlight is that the identification of a demanding growth microorganism like E. corrodens, which was previously carried out based on the type of growth on agars and through biochemical tests or 16S rRNA sequencing, was a complex and time-consuming process. Currently, MALDI-TOF mass spectrometry emerges as an effective, fast, and economical tool for the detection of demanding and slow-growing pathogens, significantly simplifying the identification process.

CO.23

HIGH RATES OF NONCONFORMITY IN THERAPEUTIC DRUG MONITORING REQUESTS: A CALL FOR IMPROVEMENT

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Background: Therapeutic Drug Monitoring (TDM) is essential to ensure safe and efficient personalized drug therapy, improving the overall quality of patient care. The sharing of precise data with the Clinical Laboratory is imperative for the correct interpretation of blood concentrations and the optimization of drug dosages. Our study aimed to assess the rates of nonconformity (NC) in clinical requests.

Methods: This study encompassed a 12-month period during which we analyzed 2143 request forms from 678 patients (median age of 65.5 years, 57.8% were male). The data collated for the pharmacokinetics software encompassed the following variables: patient weight, height, clinical information, current dosing regimen, perfusion and sample collection times. Drugs included vancomycin (90.8% of requests) and aminoglycosides (amikacin and gentamicin). Patient ages were categorized into under 18, between 18 to 65, and over 65 years. We employed univariate logistic regression to investigate the relationships between the type of antibiotic, patient age group, and the requesting department, with nonconformities expressed as odds ratios (95% CI).

Results: Analysis of the 2143 processed requests indicated that 1681 (78.6%) exhibited NCs. The most substantial NC rate (29.6%) was related to inaccuracies in documenting the start of current dosing regimen. The logistic regression identified an elevated likelihood of NC within the aminoglycosides' therapy group, particularly regarding sample collection variables [perfusion time odds ratio (OR) 2.84 (CI 2.09-3.84, $p<0.001$), trough concentration collection time OR 2.09 (CI 1.35-3.25, $p=0.001$), peak concentration collection time OR 2.23 (CI 1.61-3.09, $p<0.001$)]. For the majority of variables, with the exception of sample collection times, a linear trend indicating increased NCs was observed across age groups ($p<0.05$). Departments of Medicine presented fewer NCs when compared to Surgical specialties, except for sample collection times ($p<0.05$). Adult Intensive Care Units (ICUs) showed a reduced probability of NCs in comparison to standard medical wards, while Pediatric ICUs demonstrated even fewer errors than adult ICUs ($p<0.05$, respectively).

Conclusion: The investigation conducted at this single institution has unveiled a substantial prevalence of NCs within clinical laboratory requests. Such discrepancies have the potential to induce inefficiencies in patient management. The insights gained from this study shed light on the nature of NCs and delineate areas requiring targeted intervention.

CO.24

TRAVELLING BACK IN TIME: EXPLORING THE IMPACT ON ANALYTICAL RESULTS OF MULTI-USER PREPARATION OF LYOPHILIZED CALIBRATORS

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Introduction: The calibration of laboratory analysers allows them to carry out the quantification procedure and thus obtain analytical results, consequently becoming highly critical for the quality assessed by the clinical laboratory. On the other hand, with each calibration, a new bias is introduced into the measurement system, making it one of the biggest sources of variability in laboratory analytical systems. For this reason, laboratories prefer ready-to-use liquid calibrators, in the conviction that variability is reduced. Can the calibration alone be the only source of associated variability or can the preparation of the calibrator, in the case of being lyophilized (LYO), be the greatest enemy of bias and imprecision?

Objective: The aim of this study is to assess the impact in the results of multiple users on the preparation of a chemistry multiparametric LYO calibrator, and the impact of the calibration itself.

Materials and methods: 10 users prepared each, 1 LYO calibrator (Abbott® Consolidated Chemistry Calibrator). A calibration was performed with each calibrator and 2 control levels were used, for the assays: Creatinine (CRE), Cholesterol (CHO), Total Proteins (TP) and Iron (IR). Additionally, 2 independent calibrations were performed with the same calibrator (4 users). Statistical analysis used the Kruskal-Wallis and the Mann-Whitney U tests for independent samples, respectively (SPSS).

Results: For the 10 users, statistically significant differences ($p<0.05$) were found in the results for all tests and at both control levels. Minimum and maximum mean values between users were: 110.2-117.6 mg/dL (CHO); 0.77-0.81 mg/dL (CRE); 3.72-4.03 g/dL (TP) and 76.9-82.9 µg/dL (IR), for level 1 (n=20x10) and 271.6-288.7 mg/dL (CHO); 6.40-6.81 mg/dL (CRE); 6.73-7.30 g/dL (TP) and 245.2-265.0 µg/dL (IR), for level 3 (n=20x10). In the 2 independent calibrations the results revealed no statistical differences ($p>0.05$) in 25% to 50% of the opportunities for both levels.

Conclusions: Different users in the LYO calibrator reconstruction have an impact on the results, which is necessarily reflected in variability and analytical performance quality. Calibration alone can introduce a new bias in the results. Our research suggests that it is preferable to avoid the use of LYO calibrators but, if this is not possible, at least limit the number of users to reduce variability in the analytical system.

CO.25

A CASE OF SPLENIC MARGINAL ZONE LYMPHOMA HIDDEN BY AUTOIMMUNE HEMOLYTIC ANEMIA

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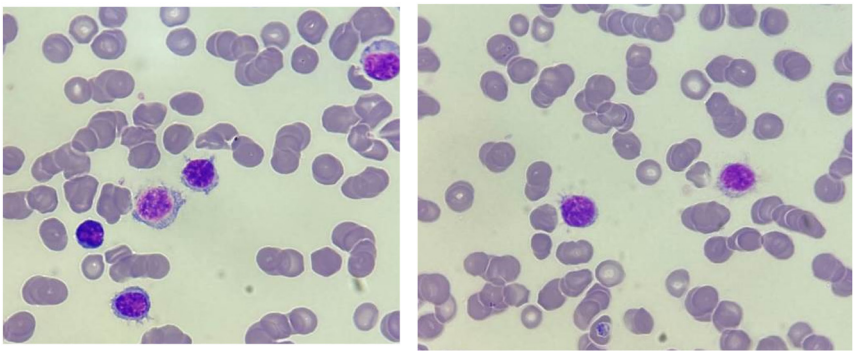
Introduction: The splenic marginal zone lymphoma (SMZL) subtype incidence is about 10% of all marginal zone lymphomas, which account for about 6% of all B-cell lymphomas. They are indolent and differential diagnosis is not

straightforward. This is case presenting with recurrent autoimmune hemolytic anemia (AIHA) which confers poor prognosis and serves as a marker of disease activity that should be considered during risk stratification of patients.

Case presentation: A 78-year-old woman is referred to Internal Medicine (IM) by her primary care physician due to pancytopenia with description of atypical lymphocytes, weight loss and splenomegaly, she had been diagnosed 2 years before with AIHA of mixed type (IgG, IgM and C3), so IM presumptively assumed the diagnosis of splenomegaly secondary to AIHA and a possible lymphoproliferative neoplasm. After splenectomy, the immunophenotyping of peri-hilar lymph nodes were CD20+, BCL2+, CD5-, C23-, cyclin D1- e CD10-, CD3-. Findings compatible with splenic marginal zone lymphoma.

Discussion: This case makes us think which of these diseases developed earlier and how they are related. It probably was the SMZL but because it is more insidious, it was diagnosed much later. The AIHA is in fact a symptom, a consequence of SMZL. This case is especially important to highlight the need to investigate further when the patient presents with abnormal hematological findings and splenomegaly, as well as the multidisciplinary approach for optimal patient care.



CO.26

DOES THE H63D MUTATION HAVE A SIGNIFICANT IMPACT ON THE PEOPLE WHO HAVE IT? OR IS IT JUST ANOTHER CLINICAL "LABEL"?

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Introduction: Mutations in HFE gene can predispose to increased iron absorption and consequent deposition in the tissues. However, not all known mutations have the same prognosis, as is the case of the H63D mutation, which compared to the C282Y mutation, presents a benign behavior like general population.

Objectives: Clinical and analytical characterization of patients referred for hyperferritinemia with and without H63D mutation. Investigation of possible parameters with significant differences between the two groups.

Methodology: Retrospective study of the last 5 years in the Internal Medicine consultation dedicated to hyperferritinemia and hereditary hemochromatosis. Patients with C282Y mutations or compound with this were excluded because their recognized greater risk. Patients were first analyzed in 2 groups (wild-type WT vs H63D mutation), and then into 3 groups by subdividing the mutated into heterozygous (H63D_HT) and homozygous (H63D_HM).

Results: 122 patients were selected for this study, of which more than 80% were men. At referral mean age was 54 years (±13 years). Over 70% were overweight (n=58) or even obese (n=31). The majority deny smoking habits (57.4%) and alcohol abuse (60.7%). 30% had a family history of hyperferritinemia and 32% had malignant neoplasia background. Only 2 patients had a personal history of cancer. Exactly 50% had the H63D mutation, 47 heterozygous and 14 homozygous. In the initial analytical

study, no statistically significant differences were found between WT patients and those with the H63D mutation in hemoglobin [15,32g/dL (±1,56) vs 14,98 (±1,38)], ferritin [948ng/ml (±743) vs 894 (±795)], serum iron [120ug/dL (±40,7) vs 132 (±48,2)] and ST index [40,8% (±16,7) vs 44,1% (±16,7)]. The absence of differences remained in the analysis with 3 groups. At the 2nd consultation, only ferritin showed a decrease, and this was more pronounced in patients without mutation (WT 948 to 671ng/ml; H63D mutation 894 to 843ng/ml; H63D_HT 922 to 912ng/ml; H63D_HM 796 to 611ng/ml). But unfortunately, no statistically significant differences were identified in the analytical parameters from the 1st to the 2nd consultation. No associations were found between habits and lifestyles and the presence of the H63D mutation.

Conclusion: This study gives credence that the presence of the H63D mutation is not associated with relevant differences to the general population, therefore, it does not require special care or even differentiated monitoring.

CO.27

WHEN THE LABORATORY TAKES THE REINS OF DIAGNOSIS

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Introduction: Monoclonal gammapathies are relatively common. Its most severe form - Multiple Myeloma (MM) - is the second most common haematological neoplasm and there are around 500 to 700 new cases a year in Portugal.

This work describes two clinical cases that highlight the proactive role that Clinical Pathology (CP) can play regarding the diagnostic process.

Clinical Case(s): The first case concerns a 66-year-old, autonomous woman who came to the emergency department with asthenia, anorexia, weight loss and hypersudoresis that had been going on for three weeks, and had been medicated with non-steroidal anti-inflammatory drugs (NSAIDs). Analytically, she had anaemia (haemoglobin (Hb) 9.9 mg/dL) with mild thrombocytopenia, grade III acute kidney injury with serum creatinine of 5.3 mg/dL (baseline 0.6) and slightly increased C-reactive protein (13.5 mg/dL). An abdominal-pelvic computed tomography showed no alterations. The initial hypothesis was viral infection (positive test for Influenza B virus) associated with acute interstitial nephritis caused by NSAIDs, so corticoids were started. In the CP laboratory, the peripheral blood smear (PBS) showed erythrocyte rouleaux formation, and the analytical study was extended, revealing a markedly increased sedimentation rate (93 mm/h), a peak in the gamma fraction (M protein of 1.11 mg/dL) and increased IgA with immunoparesis. These results led to urgent evaluation by Clinical Haematology (CH) and to MM diagnosis confirmation.

The second case concerns a 72-year-old, autonomous woman who underwent a routine analytical study at the primary care request, which revealed anaemia (Hb 7.9 mg/dL), with erythrocyte rouleaux formation and plasma cells observed in the PBS. The study was extended and revealed hyperproteinaemia (12.5 g/dL), a monoclonal IgG/*lambda* peak with M protein of 6.7 g/dL and immunoparesis. The patient was referred to CH, completed the study (bone marrow with 40% plasma cells and high-risk cytogenetics) and underwent treatment with a very good response, currently being monitored.

Discussion: The two clinical cases highlight the active role that a laboratory service can play in patient management, including primary healthcare patients. Interpretation of analytical results and immediate additional investigation can shorten the diagnostic process, with an important impact on prognosis.

|Sessão Comunicações Rápidas

CR.01

MICOBACTERIUM CHIMAERA: A NEW THREAT? – CASE REPORT

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Introduction: *Mycobacterium avium complex* comprises several nontuberculous mycobacteria, including *Mycobacterium chimaera*. This rare pathogen was recently discovered in this century, typically associated with heater-cooler units used in cardiac bypass procedures. A few case reports have been described of Mycobacterium chimaera in patients with underlying pulmonary disease.

Case report: A 48-year-old female, civil engineer, presented to the pneumology department complaining of hemoptysis and mucopurulent sputum. She also noted night sweats over the previous two weeks. She had a chronic pulmonary disease and she was smoker. She had no relevant epidemiological context (travels, animals, risk behaviour, contact with sick family members). There was no history of tuberculosis.

The physical examination was unremarkable. Blood counts showed Hb 13,2 mg/dL and C-Reactive Protein 0,86 mg/L. Liver and kidney function were within normal range. Thoracic computed tomography scan revealed an irregular cavitated lesion in the upper lobe of the right lung that required characterization.

A bronchoscopy was performed. The bronchoalveolar aspirate revealed acid-fast bacilli on direct examination but the *Mycobacterium tuberculosis* complex DNA was not detected. Liquid cultures were positive for *Mycobacterium chimaera*.

A diagnosis of *M. chimaera* lung infection was made, and she was treated with azithromycin (500 mg orally once daily), rifampicin (450 mg orally once daily) and ethambutol (800 mg orally once daily). She had a good clinical and radiologic outcome.

Conclusion: Due to its non-specific symptoms and insidious onset, the diagnosis of *Mycobacterium chimaera* infection is difficult, and sometimes it is misdiagnosed, leading to antibiotic or glucocorticoid overuse.

The present case highlights that *M. chimaera* tuberculosis can also occur in patients without typical risk factors, so the importance of suspecting for tuberculosis. Early recognition and treatment are essential to improving outcomes, and for this a multidisciplinary team is necessary.

CR.02

THE INCIDENCE OF LABORATORY-CONFIRMED CASES OF ENTERIC PATHOGENS IN A PORTUGUESE HOSPITAL IN LAST 10 YEARS – ONE HEALTH OVERVIEW

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Background: Only a subset of enteric pathogens is under surveillance in Portugal, and knowledge on the remaining pathogens detected in acute gastroenteritis is limited. The perspective of One Health (OH) highlights the interconnectedness and multi-sectoral nature of the human, animal, and environmental health or ill-health facets. Here, we present the ten-year incidence of all enteric pathogens diagnosed in a Portuguese hospital.

Methods: Over 10 years, from 2014 to 2023, zoonotic bacteria were isolated and identified in positive samples with *Salmonella* spp., *Campylobacter jejuni/coli/fetus*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aeromonas* spp., and *Shigella* spp. Cultural methods in specific agar medium, and identification performed using Vitek 2.0 (bioMérieux) or Maldi-Tof (Bruker).

Results: Almost the totality of zoonotic bacteria was isolated from stool samples (n = 465; 87.2%). The most common zoonotic bacteria isolates were *C. jejuni* (n = 251; 47,1%), followed by *Salmonella* (n = 202; 37.9%). On blood samples the most common was *Salmonella* spp. (n = 18; 46.2%). There has been observed an increase of identification over the years, being the higher value in 2021 (n = 104; 19.5%). Patients aged until 5 y/o were the most infected (n = 256; 48%). Zoonotic infection was more prevalent in male (n = 312; 58.5%). The hospital service where patients were more admitted was Paediatric Urgency (n = 350; 65.6%), followed by Medical Observation Unit (n = 68; 12.8%).

Conclusions: In this study, the majority of detected infections are bacterial detected in the younger and older ages, as seen in others studies. Incidence rates were affected by age, clinical setting and local test methods. Upgrading diagnostics methods increased detection rates. Pandemic period due SARS-CoV-2 lead to a delay/ reduction in zoonosis identification, and after that numbers stabilized. A cross-sectoral approach could be used for assessment of the OH capacity to detect and characterize foodborne pathogens.

CR.03

STUDY OF IN VITRO SUSCEPTIBILITY TO CIPROFLOXACIN IN URINARY CLINICAL ISOLATES OF ENTEROBACTERALES

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Introduction: The Portuguese national clinical guidance standards state that quinolones are not recommended as empirical antimicrobial therapy for urinary tract infections (UTI), as they are less effective and often promote the selection of antimicrobial resistance (AMR).

Aim: This aim of this study was to assess the *in* vitro susceptibility to ciprofloxacin in urinary clinical isolates of *Enterobacterales*.

Methods: This was a one-year, single-center retrospective study based on a convenience sampling of clinical isolates of *Enterobacterales*, including *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Proteus mirabilis* (*P. mirabilis*), obtained from midstream urine samples from men and women ≥ 18 years-old presenting with UTI symptoms, during January to December 2023. Ciprofloxacin antimicrobial susceptibility testing (AST) was carried out using the VITEK® 2 (bioMérieux) analyzer. All areas of technical uncertainty (ATU) were overcome by determining the minimum inhibitory concentration (MIC) by gradient strip diffusion or, when necessary, by downgrading the susceptibility category.

Results: A total of 1718 urinary clinical isolates of *Enterobacterales* were studied, including 1040 *E. coli*, 530 *K. pneumoniae* and 148 *P. mirabilis*. The resistance rate to ciprofloxacin in *E. coli* was 35.8% (n=166/464) in men and 63.7% (n=367/576) in women; in *K. pneumoniae* was 66.5% (n=123/185) in men and 59.4% (n=205/345) in women; and in *P. mirabilis* was 59.7% (n=37/62) in men and 53.5% (n=46/86) in women.

Conclusions: Our results reveal that *in vitro* resistance to ciprofloxacin is of concern in all *Enterobacterales* species studied, both in men and women. Notably, these data reveal a higher rate of resistance to quinolones than those reported in the Portuguese national clinical guidance standards (~30.0%). The high rates of resistance to ciprofloxacin are probably due to the misuse and overuse of quinolones over the last few decades in our country.

CR.04

ARE ENTEROBACTERALES AND PSEUDOMONAS AERUGINOSA A GROWING THREAT IN PORTUGAL? SMART 2018-21

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Introduction: In Portugal, epidemiological surveillance data on Gram-negative bacterial infections (GNBI) are scarce. The potential impact of the SARS-CoV-2 pandemic is unknown, but it is important to identify antimicrobial resistance (AMR) trends and to improve antimicrobial stewardship strategies.

Aim: The aim of this study was to characterize local epidemiology and to compare AMR tendencies in GNBI during pre-pandemic (2018-19) and pandemic (2020-21) years.

Methods: This was a retrospective study of clinical isolates submitted by Portuguese hospitals during the Study for Monitoring Antimicrobial Resistance Trends (SMART) 2018-21. Data was extracted through the SMART online platform, and EUCAST 2023 clinical breakpoints were used. Ceftriaxone and ceftazidime were used as indicator agents for third-generation cephalosporin (3GC) resistance in *Enterobacterales* and *P. aeruginosa*, respectively.

Results: Between 2018-19 and 2020-21, 386 versus 378 *E. coli*, 300 versus 318 *K. pneumoniae*, and 118 versus 189 *P. aeruginosa*clinical isolates were studied, respectively. When comparing pre-pandemic and pandemic years, there was an overall increase in the proportion of clinical isolates from the intensive care unit (ICU), namely 6.2%(n=24) to 10.8%(n=41) for *E. coli*, 17.3%(n=52) to 28.6%(n=91) for *K. pneumoniae*, and 22.0%(n=26) to 36.0%(n=68) for *P. aeruginosa*. Bloodstream infections were the most prevalent for *E. coli*, namely 32.6%(n=126) to 33.1%(n=125), and lower respiratory tract infections for *K. pneumoniae* and for *P. aeruginosa*, namely 39.0%(n=117) to 41.8%(n=133) and 71.2%(n=84) to 58.2%(n=110), respectively. There was also an increase in β-lactam resistance rates in *Enterobacterales*, and a decrease in *P. aeruginosa*, with piperacillin/tazobactam (P/T) resistance rates varying from 9.8%(n=38) to 12.7%(n=48) in *E. coli*, 53.7%(n=161) to 62.3%(n=198) in *K. pneumoniae*(p=0,031), and 32.2 (n=38) to 25.4%(n=48) in *P. aeruginosa*; 3GC resistance rates from 15.5%(n=60) to 19.1%(n=72) in *E. coli*, 52.3%(n=157) to 64.8 (n=206) in *K. pneumoniae* (p=0,002), and 33.1%(n=39) to 27.5%(n=52) in *P. aeruginosa*; and, finally, meropenem resistance rates from 4.1%(n=16) to 7.0%(n=22) in *K. pneumoniae*, and 16.1%(n=19) to 9.5%(n=18) in *P. aeruginosa*.

Conclusions: The pandemic years placed additional pressure on ICUs. Among GNBI, *K. pneumoniae* seems to be the main AMR threat in Portugal, with a sustained increase in P/T, 3GC and carbapenem resistance rates.

CR.05

BLOOD CULTURE CONTAMINATION IN A TERTIARY CARE HOSPITAL OF PORTUGAL

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Background: Bloodstream infections and their clinical outcomes contribute to substantial morbidity and mortality rates, affording significant economic consequences to Health Care Systems. The presence of viable microorganisms in the bloodstream is considered the gold standard for the detection of bloodstream infection. Therefore, blood culture represents an important tool for both the clinical and the laboratory management of this condition. However, positive blood cultures do not invariably indicate infection, as they may reflect either false-positive results or sample contamination.

Methods: To provide insight into this issue at a Tertiary Care Hospital in Portugal, we conducted a retrospective study involving blood cultures from adult patients processed in our Institution, between January 2017 and December 2022.

Results: We verified that 6,6% of the total blood cultures (36,7% of the positive blood cultures) were reported as "probable contamination", values that varied according to the origin of the samples, reaching as high as 9,2% (41,9% of positive blood cultures) when obtained in emergency services.

Our data suggests that at least one out of every three blood cultures would be dispensed to be processed by the laboratory, allowing this effort to be redirected to more valuable activities.

Conclusions: We conclude that the global proportion of blood cultures reported as "probable contamination" is above the values recommended, and that the emergency services are those where the highest proportion of contamination was found. Hence, it is pertinent to enact measures aimed at decreasing the incidence of contaminated blood cultures within our Institution.

CR.06

CRYPTOCOCCUS IN BLOOD CULTURE IN A NON-IMMUNOSUPPRESSED PATIENT

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Introduction: Infection with *C. neoformans* is called cryptococcosis and is considered an opportunistic infection. Cryptococcosis affects the lungs and sometimes the CNS, the presence in the blood culture is less common. This infection is more common in immunocompromised patients, such as HIV-positive patients, and is rare in immunocompetent patients. We present the rare clinical case of an apparently immunocompetent patient with *C. neoformans* in blood culture, with probable origin in the respiratory system.

Clinical case: Female patient, 83 years old, brought to the hospital due to dyspnea, was admitted for atrial fibrillation (AF) with rapid ventricular response and hypotension. Personal medical history: heart failure, AF, chronic kidney disease and diabetes mellitus II. The CT scan revealed areas of alveolar densification with endobronchial spread and mild bilateral pleural effusion. Amoxicillin/clavulanic acid and azithromycin were started. In the microbiological examination of bronchial secretions were isolated Pseudomonas aeruginosa and yeasts. The antibiotic therapy was changed to meropenem with clear improvement and reduction in inflammatory parameters. In the blood culture, encapsulated yeasts were observed in the Gram, and yeast growth was observed in the culture. Due to the strong suspicion of cryptococcosis, we made a PCR panel of the blood culture and *Cryptococcus neoformans* was detected. The patient died after two days.

Discussion: The Gram was very important as it was suggestive of cryptococcosis and the diagnosis was confirmed by PCR. The patient was not immunosuppressed but he had risk factors for cryptococcosis as he was over 50 years old and had pulmonary disorders.

The presence of *Cryptococcus spp.* in blood cultures must be recognized as an infection and treated to reduce hematological dissemination and unfavorable outcomes.

Declaration of conflict of interests: The authors declare that they have no conflicts of interest.

CR.07

STUDY OF IN VITRO SUSCEPTIBILITY TO FOSFOMYCIN IN ACUTE UNCOMPLICATED CYSTITIS CAUSED BY ESCHERICHIA COLI IN WOMEN

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Introduction: Acute uncomplicated cystitis (AUC) is a major contributor to the global burden of infectious diseases, leading to a substantial number of healthcare visits and antimicrobial therapy prescriptions each year. Women tend to be more affected than men, and *Enterobacterales*, particularly *Escherichia coli* (*E. coli*), are the main etiological agents. The Portuguese national clinical guidance standards advocate a single oral dose of fosfomycin as a first-line antimicrobial therapy for AUC in both non-pregnant and pregnant women.

Aim: The aim of this study was to assess the *in vitro* susceptibility to fosfomycin in AUC caused by *E. coli* in women.

Methods: This was a one-year, single-center retrospective study based on a convenience sampling of clinical isolates of *E. coli* obtained from midstream urine samples from women ≥ 12 years-old presenting in an outpatient setting with AUC, during January to December 2023. Fosfomycin antimicrobial susceptibility testing (AST) was carried out using the Kirby-Bauer method, and results were interpreted according to EUCAST 2023 clinical breakpoints, that is susceptible (S) ≤ 24 mm and resistant (R) > 24 mm. All residual colonies within the zone of inhibition were ignored, and reading was performed at the outer zone edge.

Results: A total of 2245 urinary clinical isolates of *E. coli* were included. The overall susceptibility rate was 97.5% (n=2189), remaining > 90.0% across all age groups and even reaching 100.0% (n=73) in the age group < 20 years-old. The zone diameter encompassing the highest number of clinical isolates of *E. coli* (n=457) was 30 mm.

Conclusions: Our results demonstrate that urinary clinical isolates of *E. coli* continue to be highly susceptible *in vitro* to fosfomycin, meaning that this antimicrobial agent seems to remain a robust option for empiric antimicrobial therapy of AUC caused by *E. coli* in women.

CR.08

FALSE-POSITIVE BLOOD CULTURE BY PLASMODIUM FALCIPARUM

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Introduction: The use of automated continuously monitored blood culture systems (ACMBCS) allows the detection of bacteremia and fungemia. If microorganisms are present, they will metabolize the substrates present in the culture medium and will generate CO_2.

False-positive signaling by ACMBCS has been attributed to elevated white blood cell count (WBC) or overfilled vials. In the literature, the rare cases of false-positive blood cultures attributed to *P. falciparum*, reported parasitemia and incubation, between 1.8% to 19.0% and 3.7 to 42 hours, respectively.

Case report: A patient presented to the emergency department with a history of fever, fatigue and confusion, after a recent travel to Angola. The patient denied taking any drug for malaria prophylaxis. Analytically presented: hemoglobin 135 g/L, WBC 8.6×10^9/L (reference value: $4\text{-}11\times10^9$/L), thrombocytopenia (19×10^9/L), aspartate transaminase 1233 nkat/L, total bilirubin 75.94 µmol/L, procalcitonin 47.1 µg/L. A peripheral blood film revealed the presence of young trophozoites of *P. falciparum* (parasitemia 30%). The patient was admitted to the intensive care unit and started antimalarial treatment.

Emergency department staff collected a set of blood cultures (1 BacT/Alert®FA Plus aerobic bottle and 1 BacT/Alert®FN Plus Anaerobic bottle (BioMérieux, Inc)). Eighteen hours later, the Virtuo™ BacT/ALERT automated blood-culture system (BioMérieux, Inc) signaled that the aerobic blood culture bottle was positive. A Gram-stained smear of the positive bottle contents reveals the presence of mature intraerythrocytic trophozoites of *P. falciparum*. Blood, MacConkey and Colombia NaladixicAcid agar plates inoculated with blood from the aerobic bottle failed to yield bacterial growth.

Discussion: *P. falciparum* can be responsible for false-positive blood cultures. The precise minimum inoculum of *P. falciparum* required to generate a detectable signal in ACMBCS is unknown. In this case, parasitemia was 30%.

As stated in the literature, *P. falciparum* is able of growing and maturing for at least 72 hours in blood culture medium. This hypothesis is supported in this study by the observation of mature trophozoites on Gram-stain.

It is not recommended to use such detection systems to diagnose *P. falciparum*, but laboratories should recognize the appearance of *P. falciparum* in Gram-stained blood culture specimens, as malaria can have fatal consequences and every opportunity should be taken to account for the diagnosis of malaria.

CR.09

VACCINATION'S ROLE IN PREVENTING PNEUMOCOCCAL MENINGITIS - A CASE REPORT

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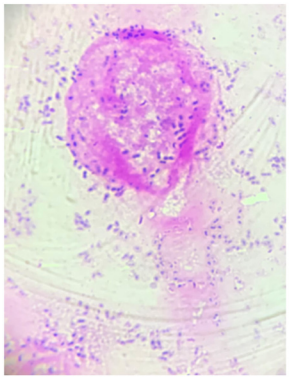
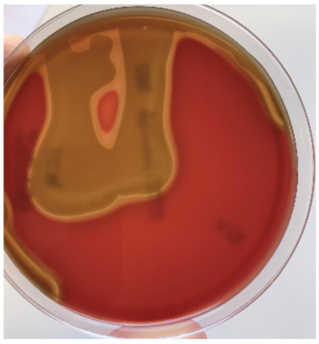
Introduction: Meningitis caused by *Streptococcus pneumoniae* continues to pose a significant concern for clinicians due to its high lethality and morbidity. It is the most frequently implicated etiological agent associated with mortality and severe sequelae in childhood.

Case presentation: A 4-month-old male infant, with no personal history, was brought to the emergency department due to a febrile illness lasting for 4 days, accompanied by irritability, and decreased nutritional intake. The analytical study revealed: anemia, leukocytosis with neutrophilia, thrombocytosis, and elevated inflammatory markers. The 4-month vaccinations are overdue. The Cytological examination revealed: leukocytosis 104 cells/mm^3 (36% polymorphonuclear and 64% mononuclear cells), glucose <2mg/dL, total proteins 266 mg/dL, leading to suspicion of bacterial infection. Following this, a panel for meningitis/encephalitis agents was performed, identifying *Streptococcus pneumoniae*. The Clinical Pathologist promptly contacted to relay the results, leading the clinician to empirically initiate vancomycin at 60mg/kg/day every 6 hours upon receiving this information. Microscopic examination of the Gram-stained smear revealed the presence of Gram-positive diplococci. After 24 hours of incubation at 37°C in a carbon dioxide-enriched atmosphere, small round alpha-hemolytic colonies with central depression, raised edges, and slight mucoid appearance were observed on blood agar. These colonies were identified as *Streptococcus pneumoniae* using the *VITEK® 2* biochemical identification system.

The antimicrobial susceptibility testing revealed sensitivity to benzylpenicillin, cefotaxime, linezolid, vancomycin, and clindamycin; resistance to trimethoprim/sulfamethoxazole. While hospitalization in the emergency department, the patient experienced a seizure episode. Subsequently, due to deterioration of consciousness, he was transferred to the pediatric intensive care unit.

Discussion: Diagnosing meningitis caused by *Streptococcus pneumoniae* in the early stages of infection proves challenging due to the manifestation of nonspecific symptoms, particularly among pediatric patients. Vaccination can potentially prevent around 70% of invasive pneumococcal infections.





CR.10

A RETROSPECTIVE STUDY OF INTESTINAL PARASITIC INFECTIONS IN A HOSPITAL CENTRE: REGARDING A CASE REPORT

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Introduction: Intestinal parasitosis (IP) is a major public health issue, especially in emerging countries. Early diagnosis is crucial due to increasing travel to and immigration from tropical countries where these infections are endemic, such as sub-Saharan Africa, America and Southeast Asia.

We describe a case report of an IP without gastrointestinal complaints and present a retrospective observational study of IP in a hospital center from 01/01/2019 to 14/02/2024.

Case report/ Case studies: A 26-year-old man from São Tomé and Príncipe, no known medical history, came to the Emergency Department with sudden and intense headache, sleepiness and disorientation. CE-CT showed a haematoma in the posterior fossa, flooding of the 4th ventricle and confirmed the presence of an AVM, originating in the left superior cerebellar and posterior cerebral arteries. He was admitted for surgery. During hospitalization, he regurgitated an adult parasite. Stool parasitological analysis was positive for *Ascaris lumbricoides*, *Trichuris trichiura*, *Giardia duodenalis* and *Schistosoma intercalatum*.

A total of 6,175 parasitological analysis were performed on 3,934 patients (median age 51.6 years; 50.1% female). 26.7% of patients aged 0-15 years were positive; conversely, only 3.0% of patients aged >15 years were positive. In the group with positive analysis, 59.8% were male (no gender differences in the negative analysis group). Upon excluding patients of Portuguese origin, 74% of the remainder were from African countries and 42.1% of these were positive for species of geohelminths, *Schistosoma* or *Taenia* spp; these species of parasites were much rarer in european patients and were found in 12.6% of them. 24.2% of african patients had 2 or more parasites identified.

Discussion: The case report and observational study show the importance of screening for IP in patients from endemic countries, regardless of clinical presentation. They are frequent carriers of medically important parasites with varying degrees of disease expression, in this sense, an accompanying careful clinical history is of the utmost importance.

In Portugal, IP research is scarce, considering the low local prevalence; but clinical suspicion must be high and, if necessary, prompt and effective treatment must be ensued – especially if followed by immunosuppressive therapy – to prevent severe and fatal outcomes.

CR.11

COAGULASE-NEGATIVE STAPHYLOCOCCUS AS AN INFECTIOUS AGENT - STAPHYLOCOCCUS UREALYTICUS

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Introduction: Most of the isolations of coagulase-negative Staphylococcus are not considered as infectious agents, but in some situations, it should be considered. We present the clinical case of a patient with isolation of *Staphylococcus urealyticus* in the pus of a wound as the possible infectious agent. *Staphylococcus urealyticus* is a rarely isolated species, coagulase negative, urease positive and resistant to novobiocin.

Clinical case: Male, 60 years old, was admitted for decompensated heart failure. With a personal history of cardiomyopathy, atrial fibrillation and gout. The patient had edema of the lower limbs with a purulent wound and inflammatory signs. Analytically in peripheral blood: leukocytes 9.90x109/L, neutrophils 9.59x109/L and C-reactive protein 102mg/L. Wound exudate was collected for microbiological examination, showing some leukocytes and many Gram-positive cocci, with the presence of intraleukocyte cocci. We isolated colonies with yellowish pigment, gamma hemolytic (without hemolysis),

positive urease and resistant to novobiocin, that was identified as *Staphylococcus urealyticus*. This microorganism was resistant to methicillin and sensitive to vancomycin, linezolid and trimethoprim/sulfamethoxazole. The patient started targeted antibiotic therapy with trimethoprim/sulfamethoxazole with improvement.

Discussion: *Staphylococcus urealyticus* is rarely isolated and sometimes is not valued because it is a coagulase negative. However, the observation of Gram was important to consider it as a pathogen. The microorganism was isolated and identified, some of its characteristics described in the literature were confirmed.

Despite being coagulase-negative, the presence of intraleukocytic cocci is strongly suggestive of infection, that's why in this case we consider the Gram analysis very important.

CR.12

CLOSTRIDIUM PERFRINGENS SEPSIS AND MASSIVE HAEMOLYSIS: CASE REPORT AND CLINICAL INSIGHT

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Clostridium perfringens, a gram-positive anaerobic bacillus, is commonly found in soil and the intestinal microbiome. Intestinal dysbacteriosis can lead to C. perfringens sepsis, a rare but fatal condition. The molecular mechanism behind its most serious complication, massive intravascular haemolysis, remains unclear. It is a rare but life-threatening condition requiring early recognition and intervention.

A 65-year-old female with a medical history including hypertension, type 2 diabetes, dyslipidaemia, and obesity, presented to the emergency department with severe abdominal pain localized in the epigastric and right hypochondrium regions, accompanied by fever.

Initial investigations suggested acute pancreatitis due to elevated lipase levels. However, within 24 hours of admission, her condition worsened rapidly. She developed worsening abdominal pain, fever, jaundice, and dark urine. Respiratory failure followed, requiring mechanical ventilation.

Laboratory tests revealed a massive intravascular haemolysis, which made it difficult to measure many of the blood parameters, and a significant drop in haemoglobin levels, all indicative of massive haemolytic anaemia. Additional abnormalities included hyperlactacidemia, markedly elevated lactate dehydrogenase levels, worsening renal function, elevated transaminases, and elevated total bilirubin. All the clinical and laboratorial findings led us to suspect C. perfringens sepsis, and targeted antibiotic therapy was initiated. CT imaging revealed liver abscesses with gas, suggesting an infected abscess.

The patient developed multiple organ dysfunction syndromes, including neurological deterioration, respiratory insufficiency, cardiovascular complications, renal failure, and hepatic dysfunction, and was transferred to the ICU for advanced management. Several days later, blood cultures confirmed Clostridium perfringens sepsis. Management involved radiologically guided drainage of liver abscesses and antibiotic therapy (piperacillin-tazobactam, penicillin).

This case highlights the rapid progression and management challenges of C. perfringens-associated haemolytic anaemia, stressing early recognition and intervention for better outcomes.

Characteristics such as massive haemolysis, and distinct erythrocyte populations (schistocytes) may indicate C. perfringens sepsis. Early recognition and intervention are vital in managing this rare but life-threatening condition, with close monitoring essential for improved outcomes.

CR.13

AN ATYPICAL CASE OF B12 DEFICIENCY ANEMIA: THE CRUCIAL ROLE OF LABORATORY TESTING IN DIAGNOSIS AND MANAGEMENT

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Megaloblastic anemia often arises from nutritional deficiencies, with vitamin B12 deficiency representing a primary example. Symptoms associated with vitamin B12 deficiency anemia may manifest as fatigue, decreased appetite, glossitis, and neurological signs including irritability, depression, and paresthesias. Findings may include elevated mean corpuscular volume (MCV), hypersegmented neutrophils, alongside low vitamin B12 levels, elevated serum lactate dehydrogenase (LDH) and decreased serum haptoglobin levels. Diagnosis relies on both clinical assessment and laboratory testing.

A 28-year-old Brazilian woman, with a recent history of depression, presented to the Emergency Department (ER) with a one-week history of frequent nausea and vomiting. She also reported a year-long history of persistent dizziness, fainting episodes, loss of appetite, fatigue, and a 6kg weight loss, exacerbated by a recent episode of a sore throat one month prior to her ER visit. Additionally, the patient exhibited signs of glossitis and reported experiencing paresthesias in her left hand. She followed an omnivorous diet.

Vital signs upon admission indicated hypotension (95/57 mmHg) and a heart rate of 76 bpm. Laboratory tests revealed a hemoglobin of 4,5 g/dL. MCV - 120 fl, white blood cells - 4,00x109/L, thrombocytopenia - 50x109/L, LDH - 1633 U/L, haptoglobin < 10 mg/dL, total bilirubin - 1,11 mg/dL, and pregnancy test negative. A peripheral blood smear showed anisopoikilocytosis, macroovalocytes, dacryocytes, schistocytes, basophilic stippling and accentuated polychromatophilia. White blood cells exhibited hypersegmented neutrophils and thrombocytopenia was confirmed.

Follow-up blood work revealed vitamin B12 levels < 100 pg/mL, with folate and iron levels within normal range. Antibodies against intrinsic factor were detected, while antibodies against antiparietal cell were not detected.

This clinical vignette illustrates a severe anemia case caused by a vitamin B12 deficiency attributable to pernicious anemia. Atypical symptoms (depression and paresthesias) along with characteristic laboratory findings in the complete blood count and blood smear should prompt the determination of vitamin B12 and folate levels, as severe deficiency can lead to permanent neurological damages, even after therapy.

CR.14

PSEUDO-PELGER-HUET ANOMALY IN A PATIENT ON RITUXIMAB

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We report the case of a 66-year-old female patient, with a previous history of Rheumatoid Arthritis, Systemic Lupus Erythematosus and Sjogren's syndrome, who presented with a routine blood count with an absolute neutrophil count of 1.44 x 10^9/L. The peripheral blood smear (PBS) was observed as part of the routine check of neutropenia cases and showed numerous hypolobated neutrophils, mainly with a single round to oval nucleus and clumped chromatin, suggestive of Pseudo-Pelger-Huët anomaly (PPHA) – Figure 1. This patient had previous complete blood count orders but no history of neutropenia and no PBS was previously required. The patient's clinical record revealed that the patient started treatment with Rituximab a few weeks earlier.

Pseudo-Pelger-Huët anomaly (PPHA) is a morphological marker of granulocytic dysplasia described in hematological diseases and in some clinical situations, including in association with certain medications. Drug-induced PPHA neutrophils are morphologically characterized by a single ovoid monolobed nucleus, significantly different from those found in cases of myelodysplastic syndrome (MDS), which are typically bilobed.

The literature shows that Rituximab can be a cause of drug-induced PPHA, which typically resolves after stopping the drug or adjusting the dose. The patient was offered to stop Rituximab treatment and a follow-up peripheral blood smear showed return of normal neutrophil morphology, confirming the drug-induced PPHA - Figure 2.

PPHA is a morphological benign condition, and therefore the distinction between an acquired iatrogenic cause or other acquired causes is of utmost importance to prevent, avoiding unnecessary investigation and invasive procedures.

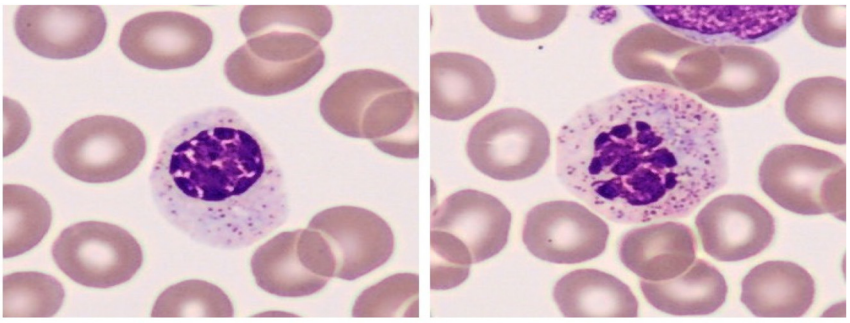

Figure 1. Non-terminally differentiated neutrophils at date of diagnosis.

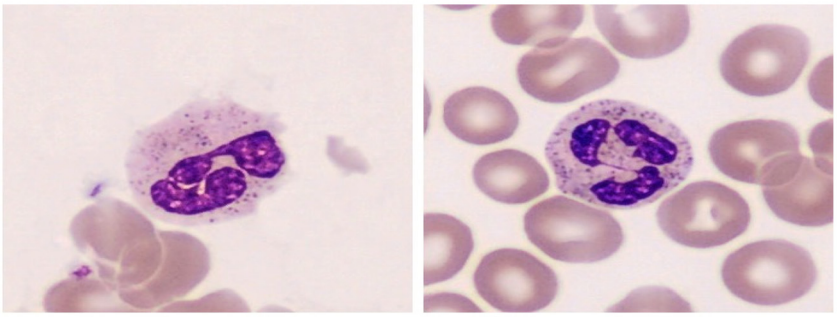

Figure 1. Non-terminally differentiated neutrophils at date of diagnosis.

CR.15

LABORATORY DIAGNOSIS OF A CHALLENGING CASE OF HIV

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Introduction: HIV-2 is characterized by lower transmissibility and lower plasma viral loads than HIV-1. HIV-2 has a slower depletion in CD4 cell counts, which may be associated to a longer asymptomatic phase and lower mortality rate than HIV-1. Also, HIV-2 is naturally resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs). HIV-2 is endemic in West Africa and Portugal is the European country with the highest prevalence of this infection. In contrast, HIV-1 has spread worldwide being responsible for the global pandemic. Besides, although co-infections are uncommon, they can occur in regions where both types co-circulate.

Here we present a possible case of an HIV-1 and HIV-2 co-infection based on the diagnostic algorithm implemented at our hospital.

Case description: Female patient, 37 years-old, native from Cabo Verde islands, living in Portugal since August 2023, was admitted to the emergency room due to a recurrent urinary tract infection. She was tested for HIV infection using the Alinity HIV Ag/Ab Combo assay (CMIA methodology). The result was positive and subsequently an imunochromatographic test for

the confirmation and differentiation of individual antigens and antibodies was performed (Bio-Rad Genius HIV 1/2). As the result was positive for all the bands of HIV 2 (gp36; gp 140) and HIV 1 (p31; gp160; p24 and gp41), RNA viral loads were performed. The viral load for HIV-2 was 21533 copies/ml (4.33 Log) and undetectable for HIV-1. The patient presented 294 cells/mm3 of CD4; the ratio CD4/CD8 was 0.3; IgG antibodies were detected for CMV and Varicella zoster virus; no specific IgM antibodies were detected. The patient was seronegative for A, B and C hepatitis virus. She started antiretroviral therapy.

Discussion: The results of the testing algorithm for HIV and the epidemiologic background, suggest a possible dual infection with HIV-1 and HIV-2. The undetectable RNA for HIV-1 is not relevant for this diagnosis since it is known that in dual-infected patients and under specific conditions, HIV-2 can inhibit HIV-1. However, this diagnosis can only be confirmed with the presence of HIV-1 proviral DNA which hasn't been tested. Finally, this clinical case emphasizes the importance of a well-defined algorithm encompassing different methodologies for a clear diagnosis of HIV infection.

|Sessão Prémio Melhor Poster

P01

URINARY FREE CORTISOL MEASUREMENT: COMPARISON OF TWO AUTOMATED IMMUNOASSAYS WITH LC-MS/MS

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Introduction: Cortisol is a steroid hormone synthesized from cholesterol in the adrenal cortex, that plays a critical role in several homeostasis actions, immune system, and stress response. Most of serum cortisol circulates bound to proteins but only the free cortisol is biologically active (<5%) associated with a circadian production. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is considered the gold standard to urinary free cortisol (UFC) determination, however, it requires an expensive equipment and skilled staff, and requires longer processing times. As an alternative, clinical laboratories may use immunoassays (IA) although some cross-reactivity with cortisol metabolites have been reported.

Objective: Compare the analytical performance and clinical agreement of the results from two different IA and LC-MS/MS to determine the concentration of UFC in 24-hour urine samples.

Materials and Methods: UFC quantification in 42 samples by: electrochemiluminescence (ECLIA-Cobas®), after extraction with dichloromethane; chemiluminescence microparticle immunoassay (CMIA-Alinity®i); and LC-MS/MS- Waters®. Results were analysed using the software SPSS® (T-test and Pearsons´s correlation).

Results: Both IA revealed good correlation with LC-MS/MS, slightly better for CMIA (ECLIA r^2=0,883; CMIA r^2=0,915). T-test showed significant statistical difference between the IA methods and LC-MS/MS. Plot analysis indicated better proximity of CMIA results with LC-MS/MS (average difference of 2,14 mg/mL vs. 5,67 for ECLIA). When the impact on clinical decision-making and the reference intervals used for each method were considered, the obtained results were consistent in 90,48% of the samples. Three samples (7,4%) had values considered pathological by the ECLIA and normal in the other.

two. One of the samples showed pathological values in ECLIA and LC-MS/MS but not in CMIA.

Conclusion: Both IA techniques quantify higher UFC values compared to LC-MS/MS, even though good correlations were observed, and good clinical concordance was found. Therefore, both techniques are suitable for diagnosis. However, it is essential to establish population-adjusted reference values, because no differences in mean values were found that could justify the large discrepancy between the reference values adopted.

References:

Antonelli G, Artusi C, Marinova M, Brugnolo L, Zaninotto M, Scaroni C, Gatti R, Mantero F, Plebani M. Cortisol and cortisone ratio in urine: LC-MS/MS method validation and preliminary clinical application. Clin Chem Lab Med. 2014 Feb;52(2):213-20. doi: 10.1515/cclm-2013-0471; Abbott and Roche cortisol test leaflets.

P02

WHEN THE LAB TAKES THE 1ST STEP: FREE LIGHT CHAIN AS THE TRIGGER TO DIAGNOSE 2 PLASMA CELL DYSCRASIA CASES

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We present two clinical cases of uncommon diseases: Light Chain Multiple Myeloma (LCMM) and AL Amyloidosis. In both, the serum free Light chain (sFLC) assay was the trigger to suspect the presence of a plasma cell dyscrasia (PCD).

Case 1: A 64-year-old male was admitted to the medicine ward due to acute kidney injury. Laboratory results revealed: anaemia with haemoglobin (Hb) 8.70 g/dL (13.00-18.00), total protein (PT) 5.1g/dL (6.6-8.7), albumin (Alb) 3.6 g/dL (3.4-4.8), high creatinine 2.10 mg/dL (0.70-1.40), low gamma fraction in serum protein electrophoresis (EPS). sFLC analysis was immediately performed by the lab, showing kappa free light chain **(KF) 18.08 mg/dL (0.33-1.94)**, lambda free light chain (LF) 1.93 mg/dL, FLC ratio (rFLC) 9.37 (0.26-1.65). Further investigation revealed Kappa LCMM.

Case 2: A 56-year-old male presented at the emergency department with progressive symptom of asthenia, tiredness at moderate efforts, and peripheral edema for 4 months. Laboratory results revealed: PT 3.9 g/dl, Alb 2.2 g/dL, Hb 15.50 g/dL, EPS with low gamma fraction, imunoparesis. sFLC analysis was immediately performed by the lab, showing KF 1.27 mg/dL, **LF 11.65 mg/dL (0.57-2.63)**, rFLC 0.11. Further investigation revealed AL Amyloidosis with multiorgan involvement.

These two types of PCD pose diagnostic and monitoring challenges, because standard laboratory tests may not detect the monoclonal protein, leading to potential delays in diagnosis or underestimation of disease burden.

This highlights that EPS combined with sFLC assay is the most valuable tool in diagnosing and monitoring PCD, and that sFLC assay is particularly important in the evaluation of PCD with lower levels of Light Chain (LC) in the blood.

Abnormally low values of the FLC ratio raise suspicion, particularly when one of the LC types is suppressed.

Low levels of total proteins and albumin may suggest the presence of amyloidosis rather than other PCD.

The synergy between lab and clinic provides better results regarding early diagnosis and appropriate management, thus improving outcomes in patients with PCD. Laboratory action when interpreting the first available data can anticipate the need for further investigation towards diagnostic.

P03

VITAMIN A ASSESSMENT: INVESTIGATING CORRELATIONS AND RATIOS FOR BETTER CLINICAL PRECISION

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Introduction: Vitamin A (VA) is an essential, fat-soluble, vitamin involved in a wide range of physiological processes, and its clinical evaluation is crucial in both deficiency and toxicity situations. Diagnosis involves clinical assessment and measurement of serum retinol levels or the ratio between VA and retinol-binding protein (RBP), with a molar ratio < 0.8 suggesting deficiency. RBP assay is described as a faster and more economical alternative. Our laboratory doses VA and calculates the VA:RBP (ratio), especially when evaluating whether a low VA translates in hypovitaminosis.

Objective: The authors aim to assess the correlation between VA and RBP, as well as the importance of the VA:RBP ratio in the correction of low VA results.

Materials and Methods: Retrospective analysis of a 2 year period. VA assays by LC-MS/MS and RBP by immunonephelometry. Descriptive statistics, normality test (Shapiro-Wilk), and correlation with respective significance test (Spearman) were performed in Microsoft Excel.

Results: The mean results of the 132 adult samples (69.7% male, 30.3% female) were: 0.44 mg/L (VA), 47.9mg/L (RBP), and 0.68 (VA:RBP ratio). In VA assays, 75% were within range, 19.7% below, and 5.3% above. Normal range VA (N = 99), showed a ratio below 0.8 in 57.6%, and a normal ratio in only 32.3% of the cases; low VA had a low ratio in 96.2% of the cases and only 1 case had a normal ratio. In high VA concentrations (N = 7), only 1 case had an above range ratio, and 5 cases had it normal. The correlation studies revealed: $\rho = 0.853$ ($p < 0.01$) for VA/RBP and $\rho = 0.533$ ($p < 0.01$) for VA/ratio. Considering those with low VA (N=26), $\rho = 0.445$ ($p < 0.01$) for VA/RBP and $\rho = 0.392$ ($p = 0.05$) for VA/ratio.

Conclusions: There was a good correlation between VA and RBP assays, though weaker for low VA values. Factors such as obesity, renal function and physiological state may influence this relationship. High variability was observed in RBP values, with most cases showing a low ratio. Conditions like inflammation, chronic pancreatitis and hepatic diseases may account for this. The ratio allowed the identification of VA underestimation in only 6 cases (4.5%). The results suggest that widening the ratio range to values lower than those described in the literature might be beneficial and that VA reflects the vitamin status better than RBP assessment. The use of RBP alone may result in inaccurate VA status assessment.

P04

HAEMOGLOBINOPATHIES – STUDY OF LABORATORY DATA COLLECTION AND MIGRATORY EFFECTS

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Introduction: Haemoglobinopathies can be classified into qualitative (haemoglobin variants) and quantitative (thalassemia) and are historically associated with certain geographical areas. The correct identification of β-thalassemia and haemoglobin (Hb) variants carriers is essential for identifying couples at risk, genetic counselling, and preventing severe forms of haemoglobinopathies.

Aim: Study data collected in the laboratory on diagnosed cases of haemoglobinopathies between 2014 and 2023, and evaluate possible associations with geographic origin of individuals and migratory movements.

Material and Methods: Retrospective evaluation of the identified cases of haemoglobinopathies analysed at Haematology/Haemoglobinopathies laboratory of INSA, IP, between 2014 and 2023. Presumptive identification was carried out using at least two different analytical methodologies and following a diagnostic analytical pathway that uses first-line and confirmatory techniques.

Results and Discussion: Between 2014 and 2023 were diagnosed 1394 cases of carriers of haemoglobinopathies in our laboratory. These carriers were predominantly females (65.4%) with a mean age at diagnosis of 35 years.

The number of samples analysed decreased sharply in 2020 due to the Covid-19 Pandemic, and then recovered partially from 2021 to 2023. The positive cases identified associated with genetic risk was 34% (2014 to 2019) and 71% (2020 to 2023). This increase is explained by the prior screening of samples sent for confirmation. There was a significant increase in the detection of Hb C and Hb E variants, mainly evident after the pandemic (2014-2020: Hb C carriers = 19.1%, Hb E carriers = 3.4%; and 2021-2023: Hb C carriers = 35.0%, Hb E carriers = 11.0%). This is most likely due to immigration flows of populations from areas endemic to these Hb variants. On the other hand, the percentage of cases of β-thalassemia and Hb Lepore carriers had only a slight increase, probably because they are autochthonous.

Conclusion: It is becoming increasingly clear that the identification of carriers of haemoglobinopathies is a problem that is not restricted to certain geographic regions previously identified as high risk, corroborating the need for a universal and inclusive screening model that can provide knowledge of the present reality of the population living in Portugal.

P05

HEMOGLOBIN VARIANTS IN THE ALENTEJO COAST POPULATION: FROM GLYCATED HEMOGLOBIN DETERMINATION TO VARIANT DETECTION

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Introduction: Glycated hemoglobin (HA1c) is used in the diagnosis and monitoring of glycemic control in patients with diabetes. The High Performance Liquid Chromatography (HPLC) method detects the presence of hemoglobin (Hb) variants, allowing for more critical analysis of the result, while other methods do not. Hemoglobinopathies, a global health problem, affect more than 70% of countries, with variants estimated to be present in 7% of the population. Of the 1800 variants described, the most common are HbS, HbE, HbC and HbD, with heterogeneous geographical distribution due to migratory phenomena. HbS is more prevalent in the African population and HbE in the Asian population. In Portugal, the most common hemoglobinopathies are HbS and β thalassemia, with heterogeneous distribution and higher incidence in the center and south.

Objective: Based on the determination of HbA1c, to evaluate the frequency of Hb variants in the population of the ULSLA.

Material/Methods: For 9 months, HbA1c was carried out on peripheral blood samples collected in EDTAK3 at ULSLA. The method used was HPLC, equipment TOSOH G8® (HORIBA). The samples were processed in variant mode - to determine HbA1c and detect variant peaks. Those with a variant peak were processed in B-Thalassemia mode - confirmation, identification and quantification of the variant.

Results: April 2023 to January 2024, HbA1c was determined in 7046 patients of various nationalities: Europe (6893 - 6773 Portuguese), Africa (21), Asia (71) and Latin America (61). Of the 7046 patients, 93 (1.32%) were found to have Hb S, E, C and D variants. Of these, 70 patients had HbS (75.3%), 12 HbD (12.8%), 6 HbE (6.5%) and 5 HbC (5.4%). Of those with HbS: 66 (94.3%) Europeans, 3 (4.3%) Africans and 1 (1.4%) Latin America; HbD: 10 (83.3%) Europeans and 2 (16.7%) Asia; HbE: 5 (83.3%) Asia and 1 (16.7%) European and HbC: 2 (40%) Europeans, 1 (20%) African and 2 (40%) Latin America.

Conclusion: This study showed that the frequency of Hb variants in the Alentejo coast region is in line with that described in the literature, as well as their global migration. The HbS and HbD variants were detected mainly in Europeans (Portugal). The distribution of HbS coinciding with that described (higher prevalence in the district of Setúbal - ULSLA area - and Beja). As described in the literature, HbE was detected in Asians (Thailand and Bangladesh).

P06

MASS SPECTROMETRY COMBINED WITH ARTIFICIAL INTELLIGENCE: ANALYSIS OF PROTEIN PROFILE OF ESCHERICHIA COLI

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Background: *E.coli* infections are major world-wide public health concern. In hospital settings, patients frequently present with infections caused by *E. coli* strains bearing different repertoires of virulence genes. This type of infection is common in the Oncology wards to due high fatality. Virulence of different *E.coli* strains can be efficiently identified by employing mass spectrometry (MS). MS and Artificial Intelligence (AI) can be used to identify proteins involved in virulence and analysis of the data, offering the capability of performing pattern recognition on uninterpretable multivariate data. Artificial Neural Networks (ANNs) are “intelligent systems” for recognizing patterns which allow the generation of data systems with an algorithm to control and evaluate infections in different wards.

Objective: Evaluate the protein profile of *E.Coli* in different hospital wards.

Materials and Methods: We carried a study in January 2024, where we evaluated the *E.coli* protein profiles in 10 immunocompromised patients of admitted to the Oncology Unit and 10 non-immunocompromised patients aged 40 years or less admitted to the General Medicine Unit. The analytical method for protein identification used was Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) using the *MALDI Biotyper® sirius - Bruker ®*. T-TEST was used to compare the significance.

Results: There is a significant difference between MS profiles in the different groups. The different observed patterns allowed the identification of a MS protein profile "fingerprint" associated with each group. We were able to delineate specific biomarkers.

Discussion/Conclusion: MS protein profiling allows the evaluation of differences in pathogenicity and virulence of *E.coli* present in different patient groups, allowing the clinician to direct therapy. Specific MS "fingerprint" were identified in different patient groups, and specific protein biomarkers can be therefore defined. MS is a convenient and accurate method to evaluate the protein profile of microorganisms in each patient, allowing to rapidly evaluate *E.coli* strains. As a follow up, an algorithm can be created using AI for the different hospitalization wards, to detect and make predictive models on widespread hospital infection and anticipating isolation measures.

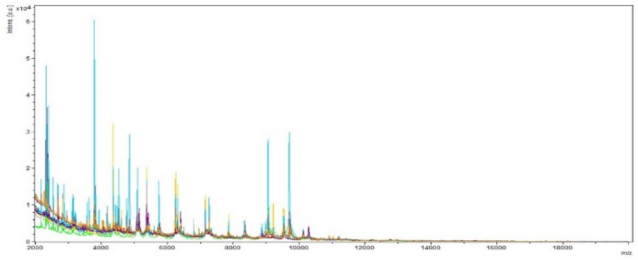

Fig.1. *E.coli* protein profiles in 10 immunocompromised patients of admitted to the Oncology Unit

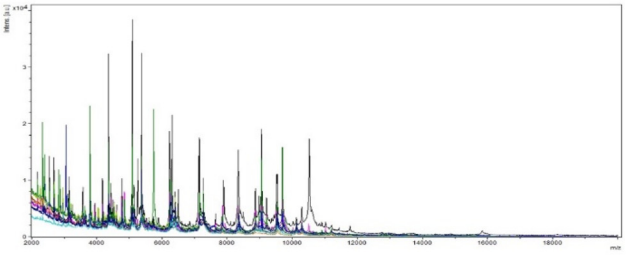

Fig.2. *E.coli* protein profiles in 10 non-immunocompromised patients aged 40 years or less admitted to the General Medicine Unit

P07

NEUROBLASTOMA: A CLINICAL CASE EVALUATED BY FLOW CYTOMETRY – DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

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Introduction: Pediatric solid tumors (PST) are a heterogeneous group of diseases that comprise 40% of all pediatric cancers. Early diagnosis and accurate classification of pediatric cancers are key for optimal therapeutic choice and adequate patient management.

Multiparameter flow cytometry (MFC) grants simultaneous evaluation of multiple proteins in millions of single cells, in a fast and accurately way. Recent reports suggest that MFC can make a valuable contribution to pediatric non-haematopoietic tumors, identifying different antigen expression profiles strongly associated with (or even specific for) some diagnostic subtypes. Co-expression of CD56+, CD90+, GD2+, CD81+, and CD9+ in the absence of CD45 is a typical immunophenotypic (IF) feature of neuroblastoma (NBL) cells.

Classical immunotherapy or chimeric antigen receptor (CAR)–expressing T cells targeting disialoganglioside GD2 are novel emerging therapeutic options for patients with high-risk NBL. We present a pediatric clinical case with NBL evaluated by MFC performing a screening tube (STOT) on a mass biopsy (MB) and on a bone marrow aspirate (BM).

Clinical case: 2-year-old boy with a left laterocervical mass, admitted to hospital with maternal notion of adynamia, groaning and occasional dyspnea. Computed tomography confirmed a cervicomediastinal mass, with thyroid and adjacent

vessels compression. Mass biopsy was evaluated by MFC and 16.94% of a non-haematologic population was identified, with an IF: cCD3- CD4- CD8- CD45- CD56++ CD81+/++ CD99- CD271-/+ EpCam- GD2++ MYOG-, with a DNA Index=1.17 and 14.22% of S phase cells. The BM sample presented 1.56% of a population with the same IF. The anatomopathologic study also revealed findings compatible with NBL.

Cervical and dorsal spine MRI and Body Scintigraphy revealed multiple bone lesions suggesting secondary involvement by NBL. These findings were compatible with the diagnosis of High Risk Neuroblastoma.

Conclusion: The MFC methodology allowed the management and staging of this high-risk NBL case. The high GD2 expression in these tumour cells, could make this patient eligible for anti-GD2 immunotherapy.

MFC may provide an important clue for complementary diagnostic studies and therapeutic guiding in PST.

P08

EPIDEMIOLOGY OF INFECTIONS CAUSED BY *PSEUDOMONAS AERUGINOSA* IN A PORTUGUESE HOSPITAL

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Background: *Pseudomonas aeruginosa* is an important pathogen causing severe healthcare-associated infections. This bacterium is intrinsically resistant to several antibiotics and has the ability to acquire new resistance genes, leading to untreatable infections and contributing to higher morbidity and mortality rates. It is therefore important to investigate the dissemination of this bacterium in the hospital setting to implement effective control measures.

Material and methods: Seventy-four *Pseudomonas aeruginosa* isolates were selected after identification using MALDI-TOF MS. DNA was extracted using the boiling method and BOX-PCR was performed to determine the genetic similarity between isolates.

Results: Nine main clusters were identified with similarities equal to or greater than 80% (C1-C9). Fourteen sub-clusters with 100% similarity were identified (SCA-SCO). SCC, SCF, SCG, SCJ, SCM, and SCN comprised isolates exclusively from outpatient care, and SCH from general surgery. SCA, SCD and SCI were composed by isolates from general surgery and outpatient care. SCB, SCE and SCL presented isolates from intensive care and outpatient care and SCO was constituted by isolates from burns unit and outpatient care. Three patients from burns unit presented two different clones, one of which was 100% similar and was present in the three patients and another one which was not related to other isolates from the same ward. In general surgery and intensive care, two patients presented isolates with 100% similarity.

Conclusion: Hospital dissemination of *Pseudomonas aeruginosa* seems to be occurring in critical hospital wards including in intensive care, general surgery and burns unit. The simultaneous detection of specific clones in hospital units and outpatient care, highlights the possibility of hospital dissemination of community strains. Infection control measures must be implemented or improved to reduce hospital dissemination of clinically relevant bacteria.

P09

DIAGNOSIS OF ASPERGILLOSIS: EVALUATION OF AN IMMUNOCHROMATOGRAPHIC TEST

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Introduction: Invasive aspergillosis is an opportunistic infection with high morbidity and mortality rates that affects mainly immunocompromised individuals. Early diagnosis is essential to improve the outcome. Traditional diagnostic methods, such as histology and culture, are time-consuming and/or invasive. Although PCR showed good sensitivity, specificity is still limited. Alternatively, the detection in serum and bronchoalveolar lavage (BAL) samples of galactomannan, a polysaccharide present in the cell wall of *Aspergillus* species, is an early, less invasive and more specific method for diagnosing these infections. Therefore, we evaluated the performance of a rapid Immunochromatographic (IC) test.

Material and Methods: In this prospective study, which was carried out between June-September 2023 in a tertiary hospital in northern Portugal, a total of 46 samples (20 BAL and 26 serum), corresponding to 36 patients were used to evaluate the Aspergillus GM Lateral Flow Assay (IMMY, Oklahoma, USA), using the PLATELIA Aspergillus Ag (Bio-Rad, California, USA), the gold standard, as a reference method. False-positive (FP) and false-negative (FN) results and agreement were assessed for 2 different cut-offs: 0.5 and 1.0 AU.

Results: Of the 46 samples tested, 29 were from intensive care units, 7 from non-intensive care units and 10 from other hospitals. Of this samples, 28% (13/46) correspond to women with an average age of 56 (39-80) and 74% (34/46) to men with an average age of 58 (5-79). Among the BAL samples, we found 1 FN and 1 FP results in the IC test, both using the cut-off of 1.0 AU. Among the serum samples, we found 2 FN results in the IC test, using the cut-off of 0.5 AU. When we increased this cut-off to 1.0 AU, these samples were negative in both methods. These values correspond to an overall agreement of 96% (44/46).

Conclusion: Despite the small sample size, our study is in line with others (Mercier T, 2021; Almeida-Paes R, 2022; Jenks JD, 2020) with larger samples, which report high agreement between the IC test and ELISA, in the serum and BAL samples. Owing to its quicker turnaround time, straightforward execution, cost-effectiveness by eliminating the need for expensive equipment, and the ability to individually test each sample without reagent wastage (unlike ELISA), this IC test has the potential to expedite and enhance the diagnosis of aspergillosis.

| Posters em Exibição

P11

CONTINGENCY PLAN: WHAT IS IT FOR?

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Introduction: As our laboratories increasingly shift away from paper and rely more on computer systems, challenges arise when these systems encounter failures. A well-defined and tested Contingency Plan (CP) is crucial, allowing Clinical Pathology Department (CPD) to maintain its responsiveness even in the absence of usual technological tools.

Objective: Emphasize importance of a well-crafted CP to effectively address interruptions in our computer information systems.

Presentation: We present the existing CP at our Hospital (CDP), showing implemented circuits, emphasizing its strengths, identifying improvement opportunities and outlining future goals.

A CP involves identifying potential crisis scenarios, assigning responsibilities, reallocating resources and specifying communication circuits.

Our CP encompasses different possible actions, depending on affected or halted system(s). Upon identifying an issue, appropriate measures are activated:

- if electronic requisitions service is inoperative, manual paper forms are used, previously made available throughout other Hospital Departments featuring predefined Emergency Analytical Profiles (EAP);

- In a Laboratory Information System (LIS) full outage, analytical requests are manually registered at the CPD. Samples are identified with pre-printed barcode labels and processed in our Laboratory Automation System (equipment chain) using a pre-configured EAP. Results are then printed and their availability is communicated.

Conclusions: The presence of a CP ensures consistent operation allowing for a timely laboratory response in urgent settings even during a crisis. The use of pre-printed labels expedites processing especially as laboratory equipment reads barcodes; furthermore, when computer communication is restored, retrospective registration in each patient's clinical record becomes feasible, facilitating documentation and preserving individual patient history.

In the future, we plan to continue carrying out training/simulations so that everyone is involved and informed about the actions to be taken in a crisis scenario.

P12

COMPARISON OF A RAPID STREPTOCOCCAL ANTIGEN DETECTION ASSAY WITH CULTURE FOR DIAGNOSIS OF STREPTOCOCCAL PHARYNGITIS

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Introduction: Lancefield group A *Streptococcus(SGA),*is one of the most important agents of oropharyngeal infection. To avoid unnecessary antibiotic prescription is recommended confirmation of SGA infection in oropharyngeal swabs using cultural methods or rapid antigen detection test (RADT)

Objective: This study aimed to retrospectively analyze incidence of SGA oropharyngeal infection detected by RADT in a population at a hospital in the Centre of Portugal and compare it to the gold standard culture.

Materials and methods: Pharyngeal swabs from 6365 patients with suspected streptococcal pharyngitis were tested. Swabs were initially inoculated on a CNA agar with 5% sheep blood agar plate and then used to perform rapid antigen test. Beta-hemolytic colonies were identified as *S. pyogenes* using conventional means. Data was collected from laboratory informatic system (LIS) database from January 2021 to December 2023.

Results and discussion: A total of 6422 specimens were cultured and tested with RADT. Results were in agreement for 1272 positive test results and 4790 negative test result. 132 specimens tested positive by culture and negative by RADT, while 228 specimens tested positive by RADT and negative by culture. Calculation of RADT sensibility and specificity came to results of 90.6% and 95.4% respectively. These results are in accordance to literature. Of the 6422 tests, 3146 were in women and 3276 in men. Concerning to age, 75 tests were performed in age under 1 year old, 6265 between 1 and 18 years old and 82 tests were performed in people between 19-100 years old.

Conclusion: Streptococcal pharyngitis presents challenges needs careful consideration of testing methodologies. Even though the RADT offer the advantage of quick results they can also have limitations, including the potential for false negatives and the inability to detect certain pathogens. Guidelines advocate for confirmation of negative RADT results with throat cultures due to their higher sensitivity and ability to detect a broader range of pathogens. However, variations in culture techniques and limitations of RADTs, such as specimen quality, collection and storage, underscore the importance of judicious clinical judgement and collaboration between healthcare providers and laboratory professionals.

P13

PREVALENCE OF CARBAPENEMASE-PRODUCING ENTEROBACTERALES (CPE) INFECTION: 8 YEARS SURVEILLANCE

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Background: Antimicrobial resistance has become a severe public health issue in recent years. The overuse and misuse of antibiotics have led to the emergence and spread of resistant strains of bacteria, making it difficult to treat infections. One of

the most significant threats to global public health is CPE infections, which have a high mortality rate when appropriate therapy is delayed or limited treatment options are available.

Aim: This study evaluates the epidemiological and genetic characteristics of CPE isolated from patients in our institution over an 8-year period.

Methods: A retrospective observational study from January 2016 to December 2023 evaluated all CPE infections (excluding duplicates). It evaluated the prevalence, carbapenemase (Cp) characterization, and distribution of infections across clinical samples of all patients. The characterization was made through the NG-Test CARBA 5 immunochromatographic test (NG-Biotech), which allows the identification of the five most prevalent Cp families: KPC, NDM, IMP, VIM, and OXA-48. Bacterial identification and antimicrobial susceptibility testing were carried out using the Vitek 2 system (bioMérieux).

Results: A total of 58 CPE were isolated: 56 *Klebsiella pneumoniae* (Kp) and 2 *Escherichia coli* (Ec), with an overall prevalence of 7.7% and 0.1%, respectively. The prevalence of Kp-CPE has increased significantly from 5.0% in 2016 to 17.6% in 2023, partially due to an outbreak of Kp OXA-48 like (OXA-181) in 2023. Ec-CPE was only detected in 2023 (N=2). KPC was the most frequent Cp (58.6%), followed by OXA-48 like (41.4%). No other Cp was detected. KPC was detected during all years, with a slight increase in the last two years (with 8 and 10 isolates respectively). OXA-48 like appeared in 2018 (N=1) and increased to 14 isolates in 2023 (due to an outbreak).

The urinary tract was the most common site of infection (N=40, 69.0%), followed by the respiratory tract (N=9, 15.5%), and others (N=9, 15.5%).

Conclusions: The present study provides insights into the epidemiology and prevalence of CPE in our institution. It confirms that the prevalence has significantly increased in the last two years. This emphasizes the importance of actively screening high-risk patients upon admission to allow early detection of colonization and to implement effective control measures.

P14

HEMOGLOBINOPATHIES IN A COMMUNITY LABORATORY

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Introduction: Hemoglobinopathies are the most common recessive monogenic disorders worldwide, resulting from gene alterations that affect the synthesis of the hemoglobin (Hb) globin chains. There are two categories: quantitative hemoglobinopathies if there is a change in the synthesis of a globin chain (thalassemia syndromes and hereditary persistence of fetal Hb) and qualitative hemoglobinopathies if there is a structural change in one of the globin chain units. The carriers can be detected through a complete blood count and the study of hemoglobinopathies or phenotype characterization using Hb A2 and Hb F quantification with HPLC and isoelectric focusing techniques. However, confirmation or genotype characterization can only be made through molecular biology techniques.

Aim: Analyse the prevalence of hemoglobinopathies in all samples studied for guided screening (with Hb electrophoresis request) and incidental findings (during the determination of HbA1c for diabetes monitoring) in 2022-2023.

Methods: We collected data from our laboratory's LIS between January 2022 and December 2023. The study included 19028 samples, with 640 requested directly and 18388 for diabetes monitoring purposes only. We performed HbA2, HbF, and Hb variants analysis using HPLC.

Results: After removing duplicates, we studied 10265 samples, out of wich 231 (2.25%) had Hb disorders, with 107 (1.04%) having qualitative hemoglobinopathies, and 124 (1.21%) having quantitative hemoglobinopathies. Of the 609 guided screening tests, 62 (10.2%) showed changes. 24 (3.94%) were qualitative: 15 with Hb S (62.5%), 6 with Hb C (25%), 2 with Hb D (8.3%), and 1 with Hb Lepore (4.2%), while 38 (6.2%) were quantitative. 21 out of the 62 alterations were found incidentally during diabetes monitoring, following a laboratory suggestion (33.9%). Out of the 9,656 HbA1c samples, 169 showed changes (1.75%), 83 had qualitative changes (0.86%), and 86 had quantitative changes (0.89%). Of the 83 samples, 52 did not yet request an Hb electrophoresis, 20 had HbS, 5 had HbC, 3 had HbD, 2 had Hb Lepore and 1 had an alfa chain variant.

Conclusions: This study highlights the significance of laboratory-provided data in screening hemoglobinopathies. Additionally, the results of a diabetes monitoring methodology can unexpectedly lead to identifying unknown carriers of hemoglobinopathies, proving helpful in such situations.

P15

ANALYTICAL AND BIOLOGICAL VARIATION LEADING METHOD COMPARISON FOR HBA1C

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Introduction: Measurement of HbA1c, is fundamental to monitor long-term glycemic control, adjust therapy, and predict the risk for the development of diabetes complications. There are different methods for measurement of HbA1c, being the reference method high-performance liquid chromatography (HPLC). When choosing an analyser among so diverse alternatives, method comparison is mandatory to find out if there is an acceptable concordance and correlation.

Objective: Our aim was to compare ion-exchange HPLC in Arkray ADAMS 8190V analyser against inhouse reference D10 Bio-Rad employing statistical tests and biologically relevant criteria obtained from the EFLM website (biological within-subject variation 1,6; confidence interval [CI] 1,3 - 2,4).

Material and methods: We compared the measurement of HbA1c in 81 samples, using correlation coefficient, straight line equation and Bland-Altman (BA) analysis, using Microsoft Excel® software.

Results: The study showed a correlation coefficient of 0,99 and the straight line equation: y = 0,89x + 5,63, demonstrating a strong positive correlation. The average difference between results was -0,63 +/- 2,18 mmol/mol HbA1c, confirmed by BA analysis. We also calculated the upper and lower limits of agreement (LoA), 4,89 and -3,64, respectively. We determined the CI for bias (0,22 to 1,03), ULoA (4,19 to 5,59) and LLoA (-4,34 to -2,94). On D10 23 samples (28,4%) had HbA1c concentrations above the reference range, while on 8190V 25 samples (30,9%), giving a potential 2,5% discrepancy in the clinical decision.

Discussion: Analysing our BA plot we can say that the bias is significant, as the line of equality is not within the confidence interval of the mean difference. In this case only biological goals could define whether the agreement interval is too wide or sufficiently narrow for our purpose. As we defined *a priori* the limits of maximum acceptable diferences (EFLM BV) we could surmise that these limits were not exceeded.

Conclusions: The average difference between the results was noticeably low in our study. The difference between the limits of our bias confidence interval is narrower than that documented by EFLM. The 95% confidence interval of the bias shows good agreement between the methods, so we can conclude that both analysers proved to be valid options for dosing HbA1c.

P16

SEVERE MALARIA CASE REPORT

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Introduction: Malaria is an infectious disease caused by parasites of the Plasmodium spp. group and transmitted through the bite of female Anopheles mosquitoes. There are approximately 200 species of Plasmodium, however, only 5 can infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. According to the WHO, 249 million cases were reported in 2022 in endemic areas. In Angola, *P. falciparum* predominates and is the main species associated with severe cases.

Case description: A 52-year-old man went to the ES 48 hours after the first visit, due to fever, headache and odynophagia. He returned from Angola a week ago and comes due to lipothymia and a seizure at home. On objective examination, he does not present meningeal signs, but disorientation and psychomotor slowing are identified, not responding to simple questions and hypotension 88/52mmHg. The patient did not perform malaria prophylaxis. The following clinical analyses stand out:

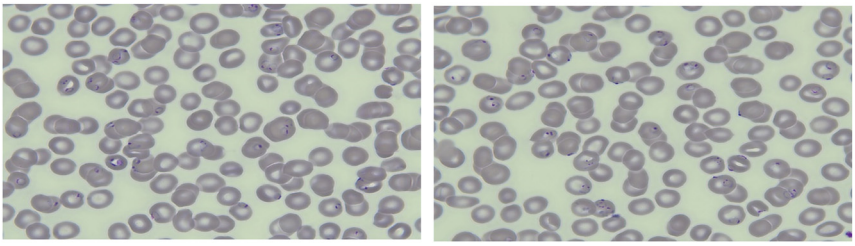
Normocytic, normochromic anemia (Hg 12.5 g/dL); Leukocytes 7.800µl; Neutrophils 75%/5.900µl; Lymphocytes 16.4%/1.280µl; Platelets 24,000/µl; PCR 19.35 mg/dL; LDH 820 U/L; Lactates 3.3 mmol/L; Kidney and Liver Dysfunction; Peripheral blood smear/Hematozoa search: 25% Parasitemia. Trophozoites suggestive of *P. falciparum* are observed. Moderate anisocytosis. Hypergranular neutrophils and presence of malarial pigment. No platelet aggregates were observed; CE-CT Scan: no changes.

Patient with Severe Malaria and Multiorgan Dysfunction is admitted to the Intermediate Care Unit and started antimalarial, Artesunate i.v., with parasitemia decreasing to 0.2% in 48 hours and becoming negative in 72 hours with improvement in clinical status, except kidney function.

Discussion: In Portugal, the last cases of autochthonous malaria were identified in 1959. However, the increase in travel from endemic countries meant that, from 2014 to 2018, 667 cases of malaria were reported. For this reason, special attention is required when observing samples from patients originating from endemic countries.

According to the WHO, the increase in the number of cases worldwide is worrying, due to a growing number of threats such as increased resistance to therapy, the impact of climate change, humanitarian crises, among other causes.

The role of Clinical Pathology in suspected cases and diagnostic confirmation stands out and requires clear and quick results.



P17

NOCARDIA ISOLATES IN A NORTHERN HOSPITAL CENTER – A RETROSPECTIVE STUDY

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Introduction: Nocardia is a delicate filamentous gram-positive branching rods that grows under aerobic conditions, it exhibits varying degrees of acid-fastness.

These bacteria can cause localized or systemic disease in humans and are typically regarded as opportunistic infections.

The most common risk factors for nocardial infection are immunocompromising conditions, environmental exposures others as chronic lung disease, diabetes mellitus or tuberculosis. *Nocardia spp* can be identified by microbiologic stains, culture or molecular testing.

Aim: The aim of this study is to present the case series of *Nocardia spp* isolates in our Hospital Center, between January 2020 and December 2023.

Results and discussion: During this time we obtained 17 isolates of *Nocardia spp* all from respiratory samples (5 of bronchial aspirate, 11 of sputum and 1 of bronchial lavage), all requested in pulmonology consultation. The main reasons to request

them were acute respiratory illness, imaging findings (study of possible neoplasia, *de novo* pulmonary structural changes or worsening of existing pulmonary changes) and follow-up of pre-existing pulmonary disease.

Patients were between 38 and 91 years old, with male gender predominance (11 men and 6 women).

Of the 17 isolates obtained, 4 different species of Nocardia were identified (*Nocardia abscessus*, *Nocardia cyriacigeorgica*, *Nocardia nova complex, Nocardia rhamnosiphila*) and in 2 situations the identification was only of genus (*Nocardia spp*).

In 6 patients, in addition to Nocardia, other microbial agents were isolated (*Aspergillus niger*, *Klebsiella aerogenes*, *Mycobacterium asiaticum*, *Mycobacterium chelonae*, *Streptococcus penumoniae*, *Haemophilus influenzae*, *Streptococcus anginosus*).

Only 4 situations (23.5%) were evaluated, by the physicians, as infection and treated with trimethoprim/sulfamethoxazole.

Conclusion: We can verify that all isolations obtained were in the context of *de novo* respiratory symptoms/chronic lung disease. With the exception of one patient, all the others had risk factors for nocardial infection. Whereas most of the isolates were evaluated as contamination/colonization (76.5%), not proceeding to antibiotic treatment.

P18

NOCARDIOSIS – A CASE STUDY

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Nocardiosis is a rare infection caused by *Nocardia spp.* The infection can have a subacute or chronic presentation, generally affecting the lung, central nervous system (CNS) and skin.

We present the case of a 70-year-old man, non-smoker, with no relevant personal history. He was admitted to the Emergency Department with a 1 – year history of coughing, oscillating chest pain, myalgias and weight loss of 4kg. He reported feverish episodes at the end of the day. On physical examination there were no changes. Computed Tomography (CT) of the chest revealed the presence of “cylindrical bronchiectasis in the middle lobe, língula and lower lobes”, with “thickening in the bronchial walls and partial occupation by bronchial secretions (SB)”. Analytically, no changes were detected, namely in the blood count, PCR, immunoglobulin levels and viral markers.

The molecular biology test for *Mycobacterium tuberculosis* was negative. Bacteriological and mycological examinations of SB were negative. The mycobacteriological culture test, carried out in MGIT liquid médium (Becton Dickinson®) was positive. From MGIT, a direct examination was carried out using the Kinyoun method, partially acid-fast bacilli were observed, with microscopic morphology suggestive of *Nocardia spp*. Subcultures were carried out in blood agar and 7H10 solid media, which showed growth of colonies with macroscopic morphology suggestive of *Nocardia spp*. Identification was carried out from these colonies by MASS SPECTOMETRY (MALDI-TOF) (Becton Dickinson®), which identified a *Nocardia abscesses*.

Given the microbiological isolation, imaging findings and the patient`s symptoms, antibiotic therapy with trimethoprim-sulfamethoxazole was initiated.

Nocardiosis is an uncommon infection that is difficult to diagnose, not only due to its varied clinical spectrum, but also due to its fastidious growth and isolation. The interpretation of positive results requires a very careful clinical and epidemiological assessment, carried out on a case-by-case basis, given the possibility that it could be an agent of colonization of the respiratory tract. Although cases of infection are mostly associated with immunocompromised patients, this infection should not be disregarded in imunocompetente patients.

P19

ACROMEGALY RECURRENCE: A CASE STUDY

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Introduction: Acromegaly is a rare and slowly progressive disease caused by the persistent excess of circulating growth hormone (GH) and insulin-like growth factor-1 (IGF-1), most often secondary to a GH-secreting pituitary adenoma. This condition typically experiences a significant diagnostic delay (10-15 years). The incidence rate is approximately 5 cases per million per year. High levels of IGF-1 are responsible for the majority of acromegaly's clinical manifestations. Serum IGF-1 levels, basal GH values, and nadir GH after an oral glucose tolerance test (OGTT) are crucial in the diagnosis, management, and treatment of acromegaly patients. The recommended diagnostic approach is to measure serum IGF-1 and, in patients with elevated or equivocal IGF-1 levels, to confirm the diagnosis by demonstrating an unsuppressed GH level of more than 0.4 µg/L following documented hyperglycemia during an OGTT using ultrasensitive GH assays. Pituitary magnetic resonance imaging (MRI) is essential for identifying the pituitary adenoma in acromegaly patients. Although transsphenoidal pituitary surgery is the first-line therapy, patients with larger and invasive tumors (macroadenomas) often do not achieve remission postoperatively.

Case presentation: A 49-year-old man was diagnosed with acromegaly at the age of 40, following progressive weight gain, sleep apnea, carpal tunnel syndrome, asthenia, and an increase in shoe size. Serum tests revealed elevated IGF-1 levels (697 ng/mL, normal range 78-184) among other hormonal imbalances. In 2017, he underwent successful surgical resection of a GH-producing pituitary adenoma, achieving biochemical remission for five years (normal IGF-1 levels and GH <0.4 µg/L). Currently, the patient is managing several comorbidities, including dyslipidemia, type 2 diabetes, obesity, hyperuricemia, hepatic steatosis, hypertrophic cardiomyopathy, severe mitral regurgitation, insomnia, depressive syndrome, heel spurs, post-surgical hypogonadism, and osteoporosis with a history of a right bimalleolar fragility fracture. A brain MRI scan in 2021 revealed a residual macroadenoma. Recent IGF-1 results (238.4 ng/mL) suggest a recurrence of acromegaly.

Discussion: In light of this clinical case, we review the pitfalls and challenges confounding the biochemical diagnosis of acromegaly. With the advent of ultrasensitive assays, current GH and IGF-1 assays, combined with new standards, have become more precise for diagnosing and monitoring acromegaly. However, their limitations must be considered alongside patient history, clinical features, and imaging when assessing for acromegaly. Managing acromegaly extends beyond achieving biochemical remission; it involves continuous monitoring for recurrence and a holistic approach to the patient's overall health.

P20

CUTANEOUS T-CELL LYMPHOMA - A CASE OF SÉZARY CELLS IN PERIPHERAL BLOOD SMEAR

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Mycosis fungoides (MF), a subtype of cutaneous T-cell lymphoma (CTCL), is characterised histologically by the proliferation of epidermotropic CD4+ T-lymphocytes with convoluted nuclei. Sézary cells may also be seen in the peripheral blood, identified by their cerebriform nuclei. Generalised erythroderma is rare and typically occurs in more advanced cases, where sepsis, especially by *Staphylococcus aureus*, is one of the most frequent causes of death.

We report a case of a female patient, 86 years old, referred to the Emergency Department for a pruritic, non-suppurative, disseminated erythematous rash. She had previously been prescribed with an 8-day cycle of prednisolone, with no improvement. No fever, signs of infection or osteoarticular pain; no pets and no perception of insect bites.

The analytical study revealed a high lactate dehydrogenase [682 U/L (135-214 U/L)], negative viral serologies and antinuclear antibodies, as well as a negative zoonotic disease multiplex-PCR panel.

No cytopenias, but a slight monocytosis and an abnormal scattergram prompted the Clinical Pathologist to perform the microscopic observation of the blood smear, which showed 4% (341/uL) lymphoid cells with variable size, convoluted/cerebriform nuclei, highly condensed chromatin and a high nuclear to cytoplasmic ratio, suggestive of Sézary cells.

These findings suggested the possibility of a cutaneous lymphoma, so the patient was admitted for further investigation. The skin biopsy showed band-like involvement of the upper dermis by a monotonous population of small to medium-sized lymphocytes with irregular nuclei, diffuse CD3, CD4 and CD5 immunohistochemical staining, negative for CD8, focal epidermotropism and occasional microabscesses, compatible with the diagnosis of MF.

Concomitantly, the patient acquired SARS-Cov-2 infection and *S. aureus* bacteremia, her condition rapidly deteriorated and she died within 10 days of hospitalisation.

Although toxicoderma was initially suspected, the Clinical Pathologist's contribution was valuable to stir the investigation in the direction of a CTCL diagnosis, as the morphological evaluation of lymphocytes on the blood smear can help differentiate patients with erythrodermic cutaneous lymphomas and erythroderma caused by inflammatory skin conditions.

P21

CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) – A RECOMMENDED BIOMARKER FOR CHRONIC ALCOHOL ABUSE

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Introduction: Transferrin (Tf) has different isoforms depending on the number of sialic acid residues attached to N-glycans chains. In healthy individuals there is a typical pattern of glycoform distribution, the most common being the tetrasialo-Tf.

This pattern can change in different clinical situations such as Congenital Disorders of Glycosylation (CDG) and liver diseases with or without excessive alcohol intake.

In chronic alcohol abuse the shift in the normal pattern is mainly due to the interference of an alcohol metabolite in the synthesis of N-glycans. This fact leads to a loss of complete chains which increases the % of the less sialized forms, asialo- and disialo-Tf (2s-Tf), known as Carbohydrate-deficient transferrin (CDT), having the 2s-Tf the best correlation with the chronic alcohol abuse. Therefore, CDT, calibrated to 2s-Tf, is the biomarker recommended by IFCC to detect chronic alcohol intake.

Aim: A 2-year retrospective analysis of CDT (IFCC) serum results obtained by Capillary Electrophoresis (minicap-Sebia®).

Materials and Methods: Data of CDT quantifications were obtained from the laboratory informatic system (Clinidata®) and analyzed using Excel® software.

Results: A total of 385 samples were performed, 156 of which belonging to adults (≥ 18 years), 28 women and 128 men; 75% of the adult results were normal (%CDT ≤ 1.7), 7.7% inconclusive (1.7 < %CDT ≤ 2.0) and 17.3% pathological (%CDT > 2.0 - compatible with chronic alcohol abuse).

The mean values for trisialo-Tf (3s-Tf), tetrasialo-Tf (4s-Tf) and pentasialo-Tf (5s-Tf) were 5.2±2.5%, 80.2±4.1% and 13.9±2.7%, respectively, in the group of adults with normal CDT results (mean = 1.2±0.3%). For the pathological group (CDT mean = 4.6 ± 2.7%) the results were: 5.7± 2.7%, 77.1±4.4% and 12.9±2.6%.

Conclusions: The data show that in parallel with the increase in %CDT there is an increase in 3s-Tf and a decrease in 4s-Tf and 5s-Tf. A higher variability for %CDT was also found in the pathological group.

A CDT (IFCC) result above 2.0% indicates, with high probability, a chronic heavy alcohol consumption. In this perspective, CDT is an important tool to detect alcoholic liver disease, however, it must always be used with precaution taking into account, namely, the values of traditional indirect biomarkers and mostly, the patient's medical history.

P22

UREAPLASMA SPECIES AND PRETERM BIRTH: A PERSPECTIVE IN PREGNANCY OUTCOMES

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Sexually transmitted infections (STIs), such as Chlamydial, Trichomonal, Mycoplasmial, and Ureaplasmial infections, are prevalent in pregnant women in many countries and are widely reported to be associated with an increased risk of poor maternal and neonatal outcomes. Published studies indicate that *Ureaplasma spp.* have been implicated in adverse pregnancy outcomes, including spontaneous preterm labor, preterm premature rupture of fetal membranes (PPROM) and clinical chorioamnionitis (1; 2; 3). Their presence in the lower genital tract has been linked to elevated levels of matrix metalloproteinases, prostaglandins, and cytokines, which are associated with the precipitation of preterm labor and PPROM (4; 5; 6). Preterm birth (defined as delivery before 37 weeks of gestation) is a significant cause of neonatal morbidity and mortality worldwide. Therefore, preventing preterm labor is a critical priority in obstetric and perinatal research.

The main objective of the present study was to identify a region-specific panel of infectious agents that can more accurately predict premature birth, as well as PPROM. To achieve this, a 3-year retrospective study was conducted. The incidence of spontaneous preterm labor, PPROM, and perinatal death (including fetal and neonatal deaths) was determined in a total of 137 pregnant women who were tested for STIs. Pathogen detection was performed using real-time PCR with the Allplex™ STI Essential Assay (Seegene). The results were analysed using IBM® SPSS® Software.

During this study, the prevalence of IST causing pathogens was as follows: 44% *Ureaplasma parvum* (UP), 22% *Mycoplasma hominis*, 17% *Ureaplasma urealyticum*, 12% *Chlamydia trachomatis*, and 6% *Trichomonas vaginalis*. Notably, UP showed higher expression levels before 28 weeks (25%) and between 33 to 36 weeks (30.9%) of gestational age. Among pregnant women infected with ureaplasma, 64% had deliveries at normal term, 19% presented preterm birth, and 17% experienced perinatal death before 21 weeks of gestation. Preterm PROM was observed in 44% of preterm births.

Our study suggests that genital ureaplasmas may be associated with various adverse pregnancy outcomes, particularly preterm birth. Further research will enhance our understanding of *Ureaplasma spp*. and their role in pregnancy and preterm birth.

P23

CRYOGLOBULIN DETECTION: PROTOCOL UPDATE AND ANALYTICAL EVALUATION IN A CLINICAL PATOLOGY SERVICE

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Introduction: Cryoglobulins (CGs), composed of immunoglobulins (Igs) or Ig complexes and complement, precipitate or gelify in serum or plasma at temperatures below 37°C, and dissolve upon reheating *in vitro*. Cryoglobulinemia is associated with various clinical symptoms such as arthralgia, purpura, glomerulonephritis, and peripheral neuropathy. Pre-analytical conditions in CG detection are crucial, with non-compliance resulting in false negatives. Lack of harmonization and absence of standardization in CG detection methods affect the reproducibility of results, with consequences to the patient.

Objectives: The authors aim to update the CG research protocol at the laboratory, by reviewing the pre-analytical and analytical processes to improve the quality of results. Results are analysed in homologous periods before and after protocol update implementation.

Materials and Methods: A literature review using PubMed database for the past 5 years was conducted. Subsequent changes to the protocol included centrifugation at 37ºC and immediate serum separation into two glass tubes, one kept cold and the second (control) reserved at 37°C, and daily observation of both, for up to a period (TAT) of 10 days. A retrospective analysis using Excel was performed on positive results after implementing updates, from 15/09/2023 to 15/02/2024, compared with the same period of the previous year.

Results: Before protocol update, 433 requests were analysed, 6 of which being equivocal for CG (1.4%) and 8 being positive (1.8%) within a TAT of up to 7 days. With the implementation of the updated protocol, 421 requests were analysed: 0% equivocal, and 52 positives were obtained (12.4%), 30 of which (7.1%) within a TAT of up to 7 days, and 22 (5.2%) between 8 and 10 days. CGs detected between the 8th and 10th day accounted for 42.3% of total positive CGs.

Conclusions: Significant increase in positive CGs was observed and the importance of extending the TAT to 10 days was confirmed. Centrifugation at 37ºC avoided precipitate losses, and the control tube reduced bias in result interpretation, contributing to better assay precision, though an external quality assessment program would help assess test accuracy. Obtaining an adequate sample volume remains a limitation. Standardizing the methodology would yield better service quality and outcomes for patients, thereby positively impacting patient safety.

P24

IMPLEMENTATION OF AN ACUTE HEPATIC PORPHYRIA SCREENING TEST IN THE CLINICAL LABORATORY – ONE-YEAR EVALUATION

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Introduction: Acute hepatic porphyrias (AHP) are a group of rare genetic diseases caused by a defect in the enzymes involved in heme synthesis, resulting in the accumulation of precursors (porphyrins), including delta-aminolevulinic acid (ALA) and porphobilinogen (PBG), which at high concentrations promote toxicity. Symptoms include severe diffuse abdominal pain, neurological dysfunction and psychiatric disorders. The severity of symptoms is variable, often misdiagnosing AHP, delaying diagnosis and onset of appropriate therapy. Therefore, a screening test in the emergency laboratory is essential to identify crisis situations and the production of toxic precursors. An example is the Hoesch screening test that is sensitive to PBG overproduction (estimated of 10 mg/L), using Ehrlich's reagent. The authors present a one-year evaluation of an AHP clinical protocol implemented in a central hospital laboratory.

Objective: Evaluate the implementation of the AHP screening procedure in a central hospital emergency laboratory, based on one-year data.

Material and methods: Urine samples were submitted to the Hoesch test; the samples were taken in crisis and kept away from light. The procedure consists of adding 2 drops of urine to 1 mL of Ehrlich's reagent and observing the color change. A reddish color indicates a positive result. PBG, ALA and total urinary porphyrins (TUP) are then measured by column chromatography to confirm the results, using a creatinine ratio.

Results: Over a one-year period, 23 urine samples - 8 men and 15 women - were tested for AHP. Only 1 sample revealed a positive result in the Hoesch test, confirmed by quantification: PBG - 16.78 µmol/mmol (reference value < 1.5); ALA (0.16 µmol/mmol) and TUP (24.03 nmol/mmol) were normal. This sample belonged to a previously diagnosed AHP case. No new cases were detected.

Conclusions: Considering the prevalence of AHP in most european countries (1/100,000) and the radius of action of the central hospital (serving around 1.8 million inhabitants), it would be expected about 18 cases. In fact, we did not study the existing cases but, in 22 suspected patients, AHP was excluded, emphasizing the relevance of this approach protocol that allows a rapid and differential diagnosis for AHP in patients in crisis.

P25

POTENTIAL ANALYTICAL INTERFERENCE IN CARDIAC TROPONIN IMMUNOASSAY

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Introduction: Cardiac troponins I and T are currently the most sensitive and specific serum biomarkers for the routine diagnosis of myocardial damage. Typically, their increases above the 99th population percentile, are caused by acute or chronic cardiac injury due to a variety of cardiac or non-cardiac pathologies, but with cardiac involvement, thus not being directly related to acute myocardial infarction.

Case Study Timeline and Events: High-sensitive troponin I (cTnI) was firstly measured (Advia Centaur XPT, Siemens Healthineers) on 19/9/23, to monitor chemotherapy induced cardiotoxity in a breast cancer patient, after the first cycle (C1) of treatment (29/8/23) with adotrastuzumab emtansine (T-DM1). [cTnI] measured was 109,29 ng/L (cutoff: £47,30 ng/L), which was re-tested after re-centrifugation. As the patient remained asymptomatic the elevation was considered nonspecific, and T-DM1 was administered. T-DM1 C3 was performed on 10/10/23 and, on 11/10/23, [cTnI] rised to 130,49 ng/L. On 31/10/23, T-DM1 cycle was not administered, and [cTnI] was 141,55 ng/L. On 21/11/23, [cTnI] fall to 80,71 ng/L, and the clinical decision was to perform T-DM1 C4. On 12/12/23, [cTnI] increased again to 159,32 ng/L. To assess the possibility of circulating macrotroponin, we conducted a precipitation test with 25% polyethylene glycol (PEG 6000), which showed a recovery of 24,8% (the recovery threshold as a positive test was suggested to be < 40%). The treatment was delayed, and on 5/1/24, we observed a [cTnI] fall (86,51 ng/L) with a recovery rate of 36,2%, which eventually normalized on 9/2/23, with at a [cTnI] measurement of 38,47 ng/L.

Discussion: The concomitant serum biomarkers, NT-proBNP and troponin T values remained normal throughout, and the patient remained asymptomatic. Cardiology conducted several cardiovascular imaging techniques: echocardiogram, showing preserved systolic function, angiotomography without signs of pulmonary thromboembolism, cardiac magnetic resonance imaging without alterations, and a FEVE of 58%, with no signs of ischemia or myocarditis. Persistente high cTnI values during treatment, without any findings, suggest that there may be an interference between the cTnI assay used, a 3-site sandwich immunoassay direct chemiluminometric method, and the therapeutic monoclonal antibody.

P26

ANALYTICAL PERFORMANCE SPECIFICATIONS (APS) – IS OUR LABORATORY ACCOMPLISHING THE SELECTED APS? CAN WE IMPROVE? A PRACTICAL EXAMPLE.

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Background: Analytical Performance Specifications (APS) are criteria that specify in numerical terms the analytical quality required for each laboratory test. 2014 Milan consensus defined 3 models for choosing APS and new hierarchies were defined, replacing the 5 models from Stockholm proposed in 1999. Some clinical laboratories, like ours, suggest rethinking the selection of these APS by selecting the highest level of this model if possible.

Aim: APS chosen by our laboratory for urea parameter (serum) is the model 2 of Milan's conference – biological variation (BV), minimum specification – 25,7% (consulted in march 2024). Our aim is to conclude if our chosen APS for the parameter urea is being accomplished.

Methods: We followed the pragmatic selection of APS proposed by the organization AEFA.

Firstly, we checked the global difficulty for accomplishing this target by consulting of the state of art graphics (percentage of labs that fullfill 100% of the selected APS). For BV minimum (25,7%) and desirable (17,1%) the global difficulty is low but for BV optimal (8,6%) it is high. To calculate our maximal limit of error (ML) we used the average of error (Xm), t-student test (t), number of months of external quality assessment (EQA) (n) and the standard deviation (SD). The formula utilized was Xm - (t(95,n-1)) * SD for the lower limit and Xm + (t(95,n-1) * SD for the upper limit. Our ML obtained was 10,39%. Xm was obtained from 12 months of EQA (year 2023). Coefficient of variation (CV) was obtained from 6 months of internal quality control (first semester of 2023). We calculated our total error (TE) using the formula: 1,65 * CV + |Xm|.

Results: ML obtained was 10,39% and TE was 9,24%. Comparing our chosen APS to ML (10,39%) and TE (9,24%), we can say that we are fulfilling the chosen APS (ML and TE < 25,7%). We can upgrade our chosen APS to BV desirable (ML and TE < 17,1%). However, BV optimal is not yet achievable (ML and TE > 8,6%).

Conclusion: This rational selection of APS can help each laboratory to know if they are accomplishing these quality specifications and if they can improve them.

P27

FALSE-POSITIVE TROPONIN I – A CASE REPORT OF AN ASSAY INTERFERENCE BY HETEROPHILIC ANTIBODIES

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We report a case of a healthy 31-year-old woman with a clinical presentation of myopericarditis (MPC) following a COVID19 infection. Her symptoms were ventricular premature beats (VPBs) and chest pain. Electrocardiogram showed sinus rhythm with isolated VPBs, the echocardiogram was normal and a cardiac magnetic resonance showed preserved biventricular function and suggested MPC.

Because of chronic basal elevation of troponin (Tn) at a 'plateau' level and chest pain, the patient underwent several diagnostic tests during follow-up, until a false-positive (FP) increase of Tn was suspected following the third admission to the cardiac intensive care unit.

The Tn measurement utilized in our laboratory is the Alinity i STAT High Sensitive Tn-I assay (Abbott) which methodology is chemiluminescent microparticle immunoassay (IA). Although a direct comparison should not be made between different IA and different Tn proteins, we tested some of the patient serum samples with the assay Elecsys Tn T hs (Roche) which method is electrochemiluminescence IA. Our lab results were in the order of thousands of units for Tn I versus normal values with Tn T (each according to the reference values (RV) of the specific IA). Our laboratory investigation for interference started by testing Tn after sample dilution (1:10) and secondly by testing for non-specific antibody interference (NSAI) and for heterophilic interference (HI) (Scantibodies tubes). Tn result obtained by our methodology was 2732 ng/L (RV according to the 99th percentile: <15,6). Tn result obtained after sample dilution was 2100 ng/L, after testing for NSAI was 4386 ng/L and after HI was 400 ng/L. The big difference between original results and results after testing for HI led to the conclusion of the presence of heterophilic antibodies, so many that it was not able to block all the assay interference.

HI is an underestimated and underrecognized cause of FP Tn elevation. In this case it led to anxiety, unnecessarily prolonged hospitalization time and exposure to an elevated number of imagological examinations. In patients presenting with persistently elevated Tn and low clinical suspicion of acute coronary syndrome and/or myocarditis, it is important to suspect FP Tn elevation and test for possible interferences therefore avoiding unnecessary diagnostic testing and treatment.

P28

GARDENERELLA VAGINALIS IN PERIANAL ABCESS

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Gardnerella vaginalis is a bacterium of the genital flora of women. When exists a vaginal dysbiosis from replacement of hydrogen peroxide and lactic-acid-producing Lactobacillus species in the vagina with high concentrations of anaerobic bacteria like *Gardnerella vaginalis* it causes bacterial vaginosis.

Men can also be infected through lack of condom use with an infected partner, however proliferation is rare due to the composition of the semen. There are diagnosed cases of inflammation of the glans and foreskin and related to urethritis and prostatitis.

A 50-year-old man, with a history of bulbar urethral stenosis and who underwent internal urethrotomy eight months ago without complications, presented to the emergency department due to dysuria, decreased urinary output, perianal swelling, with heat, edema and pain, in progressive growth, with four days of evolution without pollakiuria and purulent discharge. Analytically with leukocytosis (29,6x10^9/L), neutrophilia (25,24x10^9/L) and elevated PCR (17,64mg/dL). Kidney function well (Na 133 mmol/L, K 4.2 mmol/L, Cl 98 mmol/L). Positive Combur in leukocytes, proteins, blood and ketones. A pelvic CT scan was performed, confirming an organized multiloculated liquid collection that extends anteriorly to the root of the penis and scrotum and subsequently contacts the left anterolateral aspect of the anus, compatible with abscess formation. Adjacent and deeper, another abscess at the level of the scrotum and perianal region reveals a local inflammatory process – cellulitis.

Abscess drainage was performed. Microbiological analysis revealed the presence of *Gardnerella vaginalis*.

Antibiotic therapy was performed with favorable evolution, pain complaints were controlled and diuresis remained unchanged.

In men, the presence of *Gardnerella vaginalis* in the urethra is asymptomatic, but the bacteria can assume a pathogenic role by extending it to the prostate and bladder, especially in patients who have undergone some urological manipulation.

P29

CRYSEOBACTERIUM INDOLEGENS, IN A PATIENT WITH PERSISTENT AIR FISTULA

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Cryseobacterium indolegens is a bacteria gram negative, aerobic nonfermentative rod that can cause opportunistic infections in humans. Most infections are nosocomial and acquired through contaminated devices.

Man, 74 years old, with a history of idiopathic pulmonary fibrosis, admitted to the emergency room with left chest pain radiating to the neck, with sudden onset and pleuritic characteristics. Analytically without inflammatory parameters, a secondary spontaneous pneumothorax was diagnosed. A drain was placed and surgery was proposed to close the persistent air fistula.

Microbiological analysis of the fistula drainage revealed the presence of *Cryseobacterium indolegens*. Due to the high rate of intrinsic resistance to broad-spectrum antibiotics of this bacterial agent, there was a new surgical intervention a week later and targeted therapy with trimethoprim-sulfamethoxazole.

Given the increase in the number of cases reported in the literature related with *Cryseobacterium indolegens* like bacteremia, pneumonia, myositis, we can consider an emerging bacterium that clinicals have to deal and to have management strategies.

P30

PLASMODIUM SPECIES: AN OVERVIEW IN A PORTUGAL HOSPITAL CENTER

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Background: Malaria is an infectious disease transmitted through the bite of mosquitoes of the *Anopheles spp*. genus that affects humans and other animals and is caused by an intracellular protozoan parasite of the *Plasmodium spp*. genus. Although there are several species of this parasite, only five have been recognised as having the capacity to infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*. *P. falciparum* is the deadliest and is mainly distributed on the African continent. Early diagnosis of the disease is important to adopt the correct therapeutic measures.

Objective: Retrospective analysis of the clinical and epidemiological characteristics of patients tested positive for *Plasmodium spp.* in a hospital center.

Material and methods: The analytical method used was peripheral blood extension, on the Sysmex SP-50™ equipment. Abbot's BinaxNOW™ Malaria kit, which is an *in vitro* immunochromatographic assay, was used as the initial screening method. The T-TEST was used to compare whether the values obtained were significant or not.

Results: Retrospective study from 2020 to 2023, about the data of patients of a hospital center, who were requested to be tested for *Plasmodium spp*. There were 99 requests for *Plasmodium spp.* testing; 68 males and 31 females. The average age was 45.50 for men and 52.85 for women. From the microscopic observation of the peripheral blood extension,14 male and 3 female patients tested positive. In some cases, the study was complemented to confirm the species by classical molecular biology. The most prevalent Plasmodium species was *P. falciparum*. One sample was positive for *P. vivax*. Of the cases identified as positive, all were European with the probable place of infection in Angola. Using the T-Test, a significant p-value of P=<0.05 was obtained.

Conclusion: Globalisation and prolonged stays in endemic areas will certainly contribute to the increase in malaria cases. In this context, there is an urgent need to improve the means of diagnosis, treatment and above all, to promote traveller's meetings for the institution of prophylaxis and advice on preventive measures for infection. Diagnosis should be based on clinical history and confirmation by microscopy (to quantify parasitaemia, and it may be necessary to use molecular biology techniques to provide timely treatment). *P. falciparum* is the most diagnosed species, and the overall prevalence of the disease has decreased in recent years, as have the associated deaths.

P31

RHEUMATOID FACTOR: IS WALLER-ROSE TEST STILL AN OPTION?

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Introduction: Rheumatoid arthritis (RA) is characterized by chronic *inflammation* and progressive joint erosion, being mainly an autoimmune disorder. Among key diagnostic markers, rheumatoid factor (RF) stands out. RF is an autoantibody belonging to the immunoglobulin (Ig) M, G or A classes. Elevated RF levels serve as an important diagnosis criteria. It's important to acknowledge that RF is also elevated in other autoimmune or inflammatory disorders besides RA, as well as infectious diseases or malignancy.

Objective: Evaluate the combined results of Waller-Rose (WR) and nephelometric RF (RFN) assays and the best way to approach the RF measurement.

Materials and Methods: The study included 417 serum samples, retrospectively studied from 1st January 2020 to 15th February 2024. The WR and RFN parameters were assayed simultaneously. WR was determined by an indirect agglutination test - WR from MonlabTest®, with a Limit of Detection (LoD) of 8 IU/mL and RFN was determined by nephelometry assay - Kit FR Látex N from Siemens Healthineers at Atellica® NEPH630, with LoD <0,441 IU/mL. Limits of jaundice, haemolysis and lipemia were respected. All statistical analyses were performed by the *SPSS* software, using *Crosstabs Chi-square test*.

Results: The WR assay had 20 positive (4.8%) and 397 negative (95,2%) serum and RFN assay had 33 positive (7.9%) and 384 negative (92,1%) serum. Comparing WR/RFN: 6 serums were positive on both assays; 14 serums were positive for WR and negative for RFN; 27 serums were positive for RFN and negative for WR and 370 serums were negative for both.

Discussion/Conclusion: It is well known that nephelometry is more sensitive than agglutination. Regardless, agglutination assay is cheaper but more operator-dependent, which can, in part, explain the number of positive results alongside negative results of RFN (70% of all WR +). Only 30% of serums (from all WR+) were equally positive for both tests, while 27 serum have positive RFN and WR negative. Results correlate among themselves, but also indicate possible misinterpretation and low specificity of WR. Although cancer population has several disruptions of immunity system, in face of these results, the

advisable way to approach RF would be to perform RFN, hopefully avoiding false negative results, which could mistakenly stop diagnosis of RA or other disorders that elevate RF.

P32

CONSEQUENCES OF THE PANDEMIC CAUSED BY SARS-COV2 IN THE PORTUGUESE POPULATION

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Introduction: The Pandemic caused by the Sar-CoV2 virus began in March 2020, and caused many economic and social losses all over the world. Health systems had difficulties responding to patients and the lack of knowledge about this virus and its behavior led to deaths and extensive losses. It will certainly be important to study the consequences of this period on the health of the population.

Objectives: The main objectives are to verify how the pandemic caused by Sars-CoV2 had implications for people, both in terms of their health and on a social and psychological level.

Methods: The information was collected through a Google Forms survey with the title “Infection by the Sars-CoV2 virus (COVID-19)” distributed via email or social networks between 07/28/2023 and 01/28/2024, with a quick and voluntary response. Frequency indicators were used to analyze the data in the Excel program.

Results: 718 surveys were collected, of which 608 (85%) reported being and having been infected with the Sar-CoV2 virus. Approximately 30% (183) describe post-COVID symptoms that they associate with this infection. The symptom that stands out most is tiredness, described by 125 (68%) of these people, followed by muscle pain and loss of memory and concentration. Most respondents think that COVID 19 has had implications on their lives: 107 (17.6%) think that the work area was the most affected; 106 (17.4%) had emotional implications; 78 (12.8) think that the social area was the most affected and 59 (9.7%) feel psychological changes.

Discussion/Conclusions: We can conclude that Sars-CoV2 and the pandemic it caused left consequences on the population. A large number of people present physical symptoms that persist even after the infection. COVID 19 had consequences especially at the labor level because the period of confinement bankrupted many businesses or at least changed the routine they were used to. On an emotional and social level, the consequences were mainly due to the separation/isolation it caused. These changes could be reflected in the mental health of the population, which must be monitored over time.

P33

ANALYTICAL PERFORMANCE SPECIFICATIONS IN THE CLINICAL LABORATORY: WHERE DO WE STAND FOR TOTAL ERROR ADMITTED IN PORTUGAL?

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Introduction: Concern for patient safety and the possible impact of the results on clinical decisions have headed clinical laboratories over the last few decades to seek alignment and standardization of analytical quality. In 1999 the Stockholm Conference took the first step of commitment for a global consensus on the setting of analytical performance specifications (APS). In 2014, the Milan conference

(EFLM) defined a 3-level hierarchy, based on (1) clinical outcome, (2) biological variation (BV), (3) state of the art, and a working group was born for the setting of a database on BV. Previously, the information available on BV specifications did not always generate followers, as it was very permissive or impossible, often causing the use of state of the art, for the total error admitted (TEa). The authors present a study to evaluate the TEa(%) defined in 5 Portuguese public laboratories, considering their own concept of APS.

Objective: Comparative evaluation of the TEa% in use by the 5 laboratories represented.

Materials and methods: Comparative graphical analysis of TEa% for 9 biochemistry tests: glucose (GLU), HbA1c, creatinine (CRE), urea nitrogen or urea (BUN), cholesterol (CHOL), potassium (K), albumin (ALB), troponin I (TRP) and carbamazepine (CBZ).

Results: TEa% were: EFLM (BV) min, EMC (Spain), Rilibak (Germany), RCPA (Australia), CLIA (USA) and SEKK (Czech Republic). Only one laboratory uses ELFM BV min for all tests. Medium, minimum and maximum TEa% are, respectively: GLU:11.1/9.2/15; HbA1c: 6.8/4.7/8; CRE: 16.7/11.7/20; TRP: 20.3/20/21; K: 7.3/6/8; ALB: 8.2/4.9/10; CHOL: 10.8/8.3/13; BUN: 18.8/17.1/21; CBZ: 20/20/20. Except for K and CHOL, all the minimum TEa% correspond to the EFLM BV min. Regarding ALB and CRE, the EFLM BV min is equivalent to about half of the labs mean TEa%.

Conclusions: Most laboratories do not use BV-based EFLM APS, allowing the conclusion that laboratories continue to use different analytical quality concepts, somewhat conditioning the worldwide analytical comparison, performance and alignment. Ten years after the Milan conference, the harmonization of the APS seems not to have yet taken root, breaking the BV myth. Perhaps more time is needed, or the existence of strong national recommendations could be more effective in having a persuasive effect on the path to quality standardization.

P34

COXIELLA BURNETII SEROLOGY – A TERTIARY HOSPITAL EXPERIENCE

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Coxiella burnetii is an obligate intracellular bacterium, the etiologic agent of Q fever. Natural reservoirs include cattle, sheep, and goats. The bacteria are shed in milk, urine, faeces, and birth products. It is transmitted to humans through contact with an infected animal, consumption of unpasteurized milk or inhalation of contaminated aerosols. Most patients are asymptomatic or exhibit mild flu-like symptoms. Some patients develop pneumonia or hepatitis. It might lead to obstetric complications during pregnancy. Chronic Q fever – mainly endocarditis – requires prolonged antimicrobial therapy. Diagnostic tests are based on the detection of IgM and IgG, Phase 1 and 2 antibodies (Ab) by serological tests. Enzyme-linked immunosorbent assay (ELISA) can be used, however, the gold standard is the detection of rising Ab titers by indirect immunofluorescence (IIF). Bacterial DNA can be detected by PCR during the first week of the infection. Our objective was to evaluate the serology results obtained in our lab to optimize the diagnostic process of this rare pathogen.

Our laboratory received 305 *C. burnetii* serology test requests from a total of 279 patients during a period of 19 months. Average age was 51.7 (SD ± 19.4), 154 patients (54.0%) were male. In our laboratory, serology testing of *C. burnetii* is done by measuring phase II IgM and IgG by ELISA microtiter plates on the Triturus (Grifols) analyser. There were 38 (13.6%) positive/dubious IgM and 9 (3.2%) IgG results. Both IgM and IgG were positive in 8 samples (2.9%). In case of a positive or dubious result, we recommend repeat testing (RT) after two to three weeks to evaluate the longitudinal Ab profile. We received samples from only 13 patients (23.6%) for RT. The RT testing had the exact same serological pattern in 12 cases (92.3%). In one case, IgM was initially positive with a dubious IgG, meanwhile on RT, both IgM and IgG were positive.

C. burnetii infection is a rare and underdiagnosed disease. When suspected, a thorough medical and epidemiological history might shed light on the source of the infection. Acute disease can be detected by Phase II IgM and IgG Abs. IgM Abs are often falsely positive, therefore RT is essential to detect a rise in Ab concentration or the appearance of IgG Abs. Testing by IIF has the advantage of being able to detect phase I Abs and so diagnose chronic Q fever.

P35

THE USUAL SUSPECT – MALARIA?

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Introduction: A 59-year-old male patient was admitted to the Emergency Department with asthenia, jaundice, and a recent episode of malaria infection that took place two weeks earlier in Angola. At that time, the patient was medicated with artesunate, azithromycin and acetylsalicylic acid. He was discharged after a six-day period of inpatient care, medicated with artemether/lumefantrine, acetylsalicylic acid, and folic acid. He was reassessed 72 hours after discharge, complaining of fatigue and peripheral oedema. The lab results showed normocytic hypochromic anaemia with 7.4 g/dL haemoglobin (Hb) and slightly elevated liver enzymes. At the next re-evaluation, another 72-hours-later, he had worsening anaemia (Hb 5.9 g/dL). At this point, the patient travelled to Portugal, and from the airport came directly to the Emergency Department.

Case description: On admission, he presented with jaundice and right upper quadrant abdominal pain. The physical exam detected hepatomegaly and bilateral lower limb oedema. The lab results confirmed the anaemia (Hb 4.4 g/dL) with an elevated reticulocyte count. The liver functional tests showed elevated liver enzyme, bilirubin (total and indirect), and LDH levels. The haptoglobin level was decreased. The coagulation study showed a diminished aPTT and normal PT time. Malaria testing was requested: (1) the immunochromatographic malaria test came back positive; (2) the peripheral blood smear demonstrated anisocytosis and polychromasia, but no signs of malaria parasites were observed.

Based on the laboratory results, the presumptive diagnosis of post-artesunate delayed haemolysis (PADH) was made. The patient was admitted to the Infectious Diseases Intensive Care Unit and treatment with intravenous prednisolone was promptly initiated. He remained in the ward for five days and received multiple blood transfusions. He was discharged with rising haemoglobin and decreasing levels of cytolytic markers. Follow-up is ongoing, for the period of 12 months after the end of corticotherapy.

Discussion: We present a case of PADH, an uncommon complication of artesunate therapy. As malaria infection can also cause haemolytic anaemia, one virtually indistinguishable from PADH, a thorough laboratorial work-up is essential to adequately guide therapy and avoid a possibly dire outcome.

P36

QUALITY INDICATORS IN MYCOBACTERIUM TUBERCULOSIS COMPLEX DIAGNOSTICS FROM A TERTIARY CENTRE IN PORTUGAL, A SIX-YEAR ANALYSIS (2017-2022)

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Background: Tuberculosis (TB) diagnosis must be optimized for better patient management and the implementation of appropriate infection control and public health measures. Our micobacteriology laboratory does not have dedicated personnel to process specimens every weekday, and antimicrobial susceptibility testing (AST) is performed at the Portuguese reference laboratory due to a low number of cases. Therefore, monitoring measurable and objective quality indicators of process performance is key to assessing our working consistency over time.

Objectives: To report on five quality indicators, namely the turnaround times (TAT) for nucleic acid testing (NAAT), for sample processing to acid-fast bacilli (AFB) positive cultures, and for first-line AST results, and also report the rates for positive NAAT and smear-negative specimens in culture-confirmed TB patients. Compare those with the recommendations of the CLSI M-48 guideline.

Methods: We conducted an observational retrospective analysis of every new confirmed case of TB between 2017 and 2022. TAT's were evaluated on average days, yearly and overall, from specimen collection time for NAAT, specimen processing time for AFB positive cultures, and to first-line AST results. Rates were calculated as the percentage of the observed result in a defined period, namely 2017-2022, for NAAT positivity, and yearly and overall for smear-negative results.

Results: During the study period, 101 new cases of TB were reported (yearly range: 15-20). NAAT TAT had an average of 3 days (2-5) and 2 days for respiratory samples (67/80), with a positive rate of 76.3% total requests (61/80). TAT to achieve an AFB positive culture was 27 days (21-46) and 14 days (10-23) if it occurred in the first culture (non-contaminated) (69/101). Overall, TAT for first-line AST results was 76 days (64-93) and 64 days in the first culture. The rate of smear-negative results was 59% (50-67%; n=56/95).

Conclusion: Overall, this six-year analysis demonstrated consistency and also an improvement in trends in the quality indicators. Comparing with the CLSI M-48 guideline, the goals were achieved for NAAT TAT in respiratory samples (within 2 days), positive rate (77%) and smear-negative results (≥50%). Due to a ~30% culture contamination rate, the TAT's for AFB positive cultures (within 21 days) and first-line AST results (within 17 days of culture) were not met.

P37

BEYOND THE SKIN: UNVEILING PATHOGENICITY – A RARE CASE OF ENDOCARDITIS BY KYTOCOCCUS SCHROETERI

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Background: *Kytococcus schroeteri* is a Gram-positive coccus, part of the micrococcus branch and often encountered as part of human skin flora. It is very rarely the cause for infections in the human body but can be pathogenic in patients with prosthetic cardiac valves. We present a case of a 68-year-old patient with aortic valve endocarditis due to *Kytococcus schroeteri*.

Case Description: 68-year-old male patient presented in April 2023 with fever, fatigue and shortness of breath. Patient had history of aortic valve replacement in November 2022. Four sets of blood cultures were drawn and processed in automated blood culture system. Empirical therapy with Piperacillin/tazobactam was initiated pending culture reports. The patient was admitted to intensive care unit due to deteriorated clinical condition.

Three aerobic blood cultures bottles flagged positive for growth, thirty hours after blood collection. The gram stain showed gram positive cocci in pairs. The three positive blood culture bottles were subcultured on Columbia agar with 5% sheep blood and chocolate agar. The colonies were small yellow-white of few mm in size.

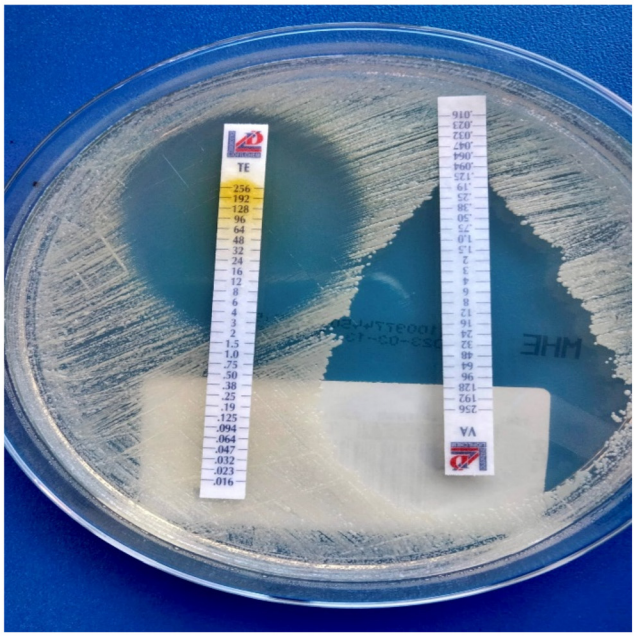
Kytococcus schroeteri was identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) in colonies from all the blood culture plates.

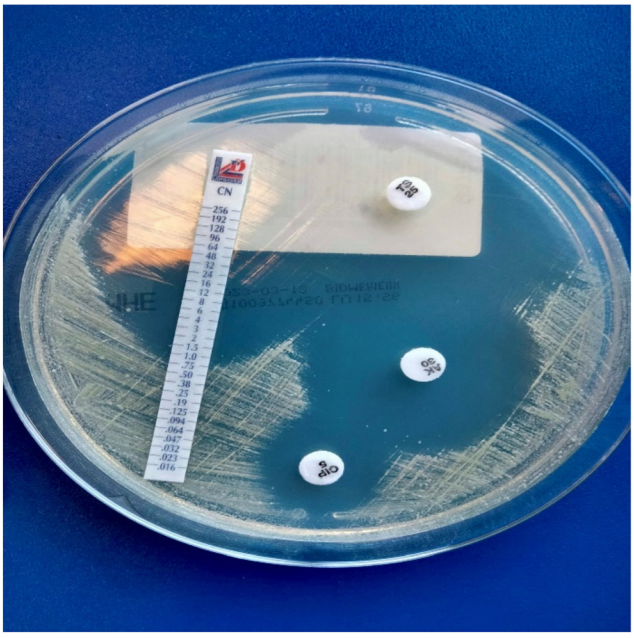
Antimicrobial susceptibility was tested by Kirby Bauer disc diffusion and gradient E-test strips methods. Since reference clinical breakpoints are not available, the decision of which antimicrobials to include in the testing and result interpretation was based on the comparison to previous cases documented in literature.

Echocardiography strongly supported infective endocarditis diagnosis. Patient was administered vancomycin, gentamicin and rifampicin with good clinical response. The aortic valve was successfully replaced. Subsequent blood cultures were negative.

Discussion: The limited knowledge surrounding *Kytococcus schroeteri's* pathogenicity, coupled with the rarity of reported human infections—primarily linked to implanted materials, notably heart valves—poses significant challenges in diagnosis and treatment. The intricate identification process and scarcity of data on antibacterial susceptibility underscore the need

for enhanced research efforts to establish standardized sensitivity data, ultimately paving the way for more effective management strategies in cases of *Kytococcus schroeteri* infections.





P38

CANDIDA AURIS – THE IMPORTANCE OF PARTICIPATING IN EXTERNAL QUALITY ASSESSMENT PROGRAM

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Introduction: *Candida auris,* identified for the first time in 2009, is an antifungal multi-resistant emergent yeast. Due to its ability to persist in a hospital environment, and to it thermo-tolerance, this species has been associated with nosocomial outbreaks worlwide. This species is difficult to identify using conventional methods and can be misidentified with other *Candida* species.

For those reasons, since 2020, the Portuguese External Quality Assessment Program (PNAEQ), with collaboration of clinical micology experts, organizes an External Quality Program (EQA) to evaluate the identification of *C. auris*.

Objective: Performance of EQA results for the identification of *C. auris* (2020, 2022 and 2023).

Methods: Participants were Portuguese public hospitals, ambulatory laboratories and one international laboratory hospital.

Nine samples with yeast species were distributed: *C. auris* (n=3) and other species of *Candida* (n=6). The other *Candida* species sent included *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis* and *C. duobushaemulonii*. Each round consisted of three samples distributed once a year.

The participants were asked to report the identification of the yeasts up to the species level and the method in use. Clinical background information of each species was provided. Results were evaluated by comparing the participant's results to the expected result.

Results: In the 3 rounds distributed, the average of enrolled participants was 3, with the exception of the pilot round, with 18 laboratories. Participants are ambulatory (42%) and hospital laboratories (58%).

Four methods were reported for species identification: automated biochemical (12/23), mass spectrometry – MALDI-TOF (9/23), non-automated biochemical (1/23) and culture – chromogenic media (1/23). Three instruments were used: Vitek 2 (12/23), Vitek MS (5/23) and Bruker biotyper (4/23) whereas two laboratories used manual methods.

Candida auris was correctly identified by 91% (21/23). Two participants reported incorrect (*C. tropicalis*)/missing answers, both used manual methods.

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

We observed a decrease in participation after the pilot study, but the participation in EQA program should be encouraged in order to perceive the above-mentioned goals.

P39

THE EVALUATING VALUE OF 3-METHOXYTYRAMINE IN PHEOCHROMOCYTOMA DIAGNOSIS: A CASE STUDY

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Pheochromocytomas and paragangliomas (PPGLs) originate either in the adrenal gland or in extra-adrenal paraganglia, arising from chromaffin cells. These neoplasms represent rare neuroendocrine tumors characterized by the production of catecholamines and can be life-threatening. Therefore, timely and accurate diagnosis of PPGLs is crucial.

Initial biochemical testing typically involves the measurement of urinary fractionated metanephrines using high performance liquid chromatography (HPLC). Elevated levels of 3-methoxytyramine (3-MT) have been observed in dopamine-producing PGLs and has been associated with an increased likelihood of metastases, particularly in the context of SDHB mutations. This case study underlines the importance of expanding diagnostic assays to include urinary 3-MT measurements.

A 34-year-old male patient was referred to the institution after presenting to the emergency department of his local hospital with a left frontal swelling, without other associated complaints. Computed Tomography (CT) revealed a left frontal swelling and a supraclavicular cervical mass on the left, suggestive of metastases. Additionally, the abdominal CT exhibit a left suprarenal necrotic area, potentially corresponding to the primary lesion. Biopsy of the cervical lymph node and adrenal analysis were requested for further investigation. The request for urinary biogenic amines analysis do not initially include the quantification of 3-MT within the panel. However, this compound was notably detected a significant peak in the chromatogram revealing markedly elevated levels of 3-MT, indicative of a potential pheochromocytoma. Dopamine and

homovanillic acid were found to be significantly increased, approximately tenfold higher than normal levels. Later, the biopsy results provided further support for the diagnosis of metastatic pheochromocytoma.

These results underscore the potential utility of 3-MT as a biomarker for assessing metastatic risk in PPGL patients, particularly in the context of SDHB mutations. Thus, incorporating measurements of urinary 3-MT into diagnostic offers a more comprehensive assessment, enhancing the accuracy of PPGLs diagnosis and enabling timely intervention.

P40

DIAGNOSIS OF A BICLONAL GAMMOPATHY – CASE STUDY

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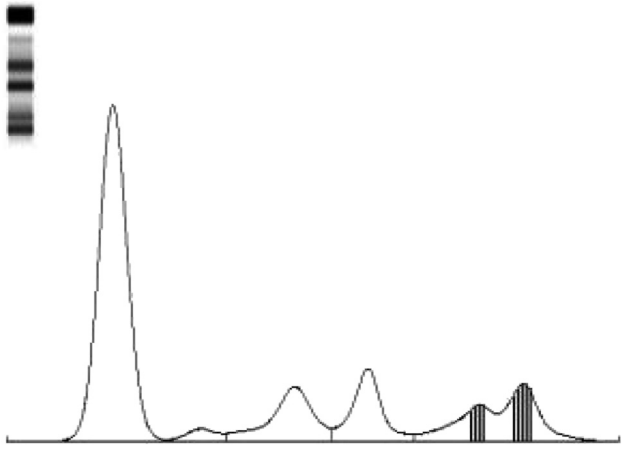
Introduction: Monoclonal gammopathy of undetermined significance (MGUS) is a clinically asymptomatic premalignant clonal plasma cell disorder defined by the presence of a serum monoclonal protein.(1) The mean age at diagnosis is 70 years old, being less than 2% of individuals diagnosed before the age of 40.(2, 3) The most common subtype of MGUS is non-IgM (mainly IgG) and it has the potential to progress to smoldering multiple myeloma (MM) or even symptomatic multiple myeloma.(1) Approximately 2-4% of individuals with MGUS have biclonal gammopathy (rather than monoclonal).

Aim: This report is about a 78-year-old female patient, referred to our hospital for persistent anemia and a biclonal gammopathy (IgAκ and λ).

Methods: Serum protein electrophoresis and immunofixation were performed, followed by bone marrow aspirate, karyotype and FISH (Fluorescence In Situ Hybridization) panel for MM.

Results and discussion: The serum protein electrophoresis revealed the presence of two monoclonal proteins in the γ-globulin zone. Immunofixation identified IgAκ and λ monoclonal chains precipitation. Within the monoclonal IgAκ peak, two related bands were observed (it may be a single polymerized monoclonal protein or a biclonal IgAκ gammopathy). The bone marrow aspirate (performed due to anemia and worsening of renal function) revealed a representative and normocellular sample, with hematopoietic cells in different stages of maturation and 1.75% of plasma cells. The patient's karyotype was 46, XX [17] and the FISH panel for MM was normal for the RB1 (13q14.2) and TP53 (17p13.1) loci and no translocations for IgH-FGFR3 t(4; 14) and IgH-CCND1 t(11; 14) were found. In the absence of MM criteria, the patient was kept in surveillance for his MGUS. Supplementation with folate and vitamin B12 were also prescribed to correct the patient's anemia.

Conclusion: This patient has an IgAκ and λ chains MGUS, a rare condition in which the risk of progression to MM is equal to a monoclonal MGUS. In the absence of criteria for disease progression (mainly significant increase in MGUS, hypercalcaemia, cytopenias, renal dysfunction or suspected/confirmed bone injury), surveillance of the patient every 3-4 months for the first 1-2 years and every 12 months thereafter is recommended.



P41

MOLECULAR DETECTION OF RESPIRATORY VIRUSES IN POST-MORTEM SAMPLES ANALYSED AFTER EXTENDED TIME INTERVALS

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Introduction: In forensic investigations, precise identification of respiratory viruses from post-mortem samples can holds paramount significance in the identification of cause of dead. Moreover, it facilitates the implementation of targeted public health measures aimed at mitigating the spread of infectious diseases within communities. Consequently, the accurate assessment of respiratory viruses in *post-mortem* samples serves as a cornerstone in both forensic pathology and public health surveillance efforts. In this work, we conducted a retrospective molecular analysis on *post-mortem* samples collected from deceased individuals.

Case presentation: Nasopharyngeal swabs were collected between 11/12/2023 and 23/01/2024, preserved under temperatures ranging from 0 to 8 ºC. Weeks later, automated RNA extraction was conducted, followed by molecular detection with real time PCR methodologies. Sample 1, obtained from an individual who passed away at the age of 75, was collected on 23/01/2024 and analyzed on 21/02/2024, reflecting a 29-day duration between collection and analysis. Notably, it yielded a positive result for Flu A. Sample 2, retrieved from an 85-year-old individual, was collected on 11/12/2023 and analyzed on 30/01/2024, a 50-day interval between collection and analysis. This sample tested positive for SARS-CoV-2. Sample 3, collected from an individual who passed away at the age of 26, was obtained on 11/12/2023 and analyzed on 21/02/2024, with a 72-day gap between collection and analysis. Remarkably, it also exhibited a positive result for SARS-CoV-2.

Discussion: These findings highlight the resilience of virus genome in swabs and the sensitivity of molecular detection methods in identifying respiratory viruses in *post-mortem* samples even after significant intervals between collection and analysis. This study highlights the importance of continued vigilance in forensic investigations, aiding in the elucidation of the epidemiology and impact of respiratory viruses in mortality cases.

P42

EVALUATION OF FAST-PREP PBC SYSTEMTM FOR BACTERIAL ID AND ANTIMICROBIAL SUSCEPTIBILITY TESTING DIRECTLY FROM POSITIVE BLOOD CULTURES

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Introduction: Sepsis, characterized by organ dysfunction due to an uncontrolled response to infection, remains a significant global health concern, with septicemia contributing substantially to morbidity and mortality rates worldwide. The traditional diagnosis of septicemia involves culture of positive blood samples, followed by microbial identification (ID) and antibiotic susceptibility testing (AST) to tailor treatment. However, conventional methods are time-consuming, delaying effective therapy and potentially exacerbating patient outcomes. The FAST-Prep™ PBC system (Qvella®) offers a promising solution, automating microbial concentration and isolation within 30 or 40 minutes.

Objective: In this study, we compared the performance of the FAST system with our routine workflow using 21 positive blood cultures (PBC) that showed to be monomicrobial with Gram-negative bacilli upon direct Gram staining.

Methods: Our routine workflow involved subculturing of PBC, on specific media. In contrast, the FAST system directly processed PBC, yielding liquid colonies. For both culture obtain, ID and AST were performed, in parallel, on VITEK MS and VITEK 2 systems, respectively.

Results: Results showed a high concordance rate between the FAST system and routine workflow for both ID (83,3%) and AST (99,5%). A few discrepancies were noted, particularly in ID, where 2 samples could not be identified and 1 showed discordant results.

Nevertheless, the overall agreement was promising, with minimal differences in susceptibility interpretations. Notably, for most antibiotics, minimum inhibitory concentration (MIC) results agreed 100%, except for piperacillin-tazobactam (85,7%), amikacin (92,9%), ampicillin (92,9%), cefuroxime (92,9%) and cefotaxime (92,9%). However, one antibiotic (cefotaxime) showed a change in interpretation according to EUCAST guidelines, with the FAST system result interpreted as increased exposure (I) and the routine result interpreted as resistant (R).

Conclusion: Despite minor discrepancies, the FAST system presents a valuable tool for expedited diagnosis and treatment optimization in patients with septicemia. Further studies should explore its applicability to other microorganisms, including Gram-positive bacteria, anaerobes, and fungi, to enhance patient care and outcomes.

P43

HOW PATHOLOGISTS PREDICT SEPSIS, WITHOUT CLINICAL FINDINGS? – NEW HEMATOLOGICAL PARAMETERS AS TEASERS OF CRITICAL CONDITION

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Introduction: Sepsis is, by World Health Organization (WHO), the most common serious complication of infection and a major cause of preventable morbidity and mortality globally. Accounts for 28%-41% of hospital mortality, affects 50 million people and causes 11 million deaths per year.

Bacterial pathogens lead cases of bloodstream infections and in spite of blood culture (BC) is the gold standard test for diagnosis sepsis, results takes among 48-72h. Early diagnosis and rapid intervention are essential, every hour delay, results in a 7-10% increase of mortality.

With acute and severe bacterial infections, neutrophils dominate the tissue response. Although there are other indicators of infection such as C-Reactive Protein (PCR) and Procalcitonin (PCT) these are later markers than Neutrophil's morphological and metabolical changes. The activation status of neutrophils (NEUT-RI, NEUT-GI), from the XN-Series can predict the appearance of "convencional" infection markers, suggesting that those can be used to detect an even earlier-stage bacterial infections.

Case report: This case reports a young woman with DRC without clinical focus of infection, after a recurrent procedure, had a normal neutrophil count with an increase intensity of reactivity of neutrophils: NEUT-RI = 79 FI (ref. 39.8 – 51.0 FI) that led to the elaboration of peripheral blood smear (PBS) showed neutrophil's changes: toxic granulation, cytoplasmic vacuoles and Döhle bodies. These observations combined indicate an early innate immune response to intracellular bacteria, to the Clinical Pathologist that recommended adding other analyses as PCR, PCT and BC. An urosepsis (BC: *K. aerogenes)* was diagnose at early stage and these parameters also helped to guide and monitor the treatment.

Discussion: This particular case emphasizes the importance of an integrated approach of sepsis's premature laboratory diagnosis. The combination of automated hematological parameters and the smear morphological analyze should be used for screening sepsis. It's a readily available and cost effective method and specific changes of neutrophils are an early marker and significantly associated with sepsis. It's a valuable tool for Pathologists detecting and monitoring a critical condition. Implementation this in routine laboratory practice will enhance the laboratory workflow, providing added-value for the Physicians, improving the out-comings and prognosis of sepsis's patients.

P44

IGD MULTIPLE MYELOMA: A RARE CASE PRESENTATION AND LABORATORY CHARACTERIZATION

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Introduction: Multiple myeloma (MM) is a malignant monoclonal gammopathy, characterized by the presence of a monoclonal protein, produced by malignant plasma cells, hypercalcemia, renal failure, anemia and bone lesions. Depending on the type of immunoglobulin (Ig) involved, myeloma can be characterized by the type of heavy chain (G, A, M, D, or E) and their respective kappa (k) or lambda (λ) light chains, or exclusively by free or heavy light chains, or even non-secretory. IgD type is a rare presentation that affects younger individuals with a typically aggressive course and worse prognosis. Due to its rarity, limited data exist in the literature, however, from a laboratory perspective, characterization is essential and should not be classified as a light chain myeloma. According to the literature, a common finding in IgD myeloma is a small or even no spike observed on serum electrophoresis, elevated Bence Jones protein, and the light chain is predominantly of the λ type. We present a case of IgD MM at our hospital which is the only one documented in 25 years.

Case presentation: A 53-year-old male with severe anemia in the context of thalassemia and a recent rib fracture. Laboratory results show haemoglobin 7.7 g/dL, creatinine 2.19 mg/dL, normal calcium levels and Igs G, A, M. Protein electrophoresis revealed a monoclonal peak of 0.79 g/dL and total proteins 7.8 g/dL. Characterization of the monoclonal peak by immunotyping revealed λ light chain.

Immunofixation confirmed the peak of λ free light chain and the peak of IgD. The measurement of free k light chain was 34.3 mg/L and λ 2280 mg/L and IgD 765 mg/dL. A 24-hours urine study revealed proteinuria of 4.2 g/24h and Bence Jones proteinuria 2.5 g/24h.

Discussion: It is worth emphasizing the importance of protein electrophoresis in detecting of the monoclonal component, with normal levels of Igs G, A, M. Using immunotyping, the detection of λ or k chains without the correspondence of the heavy chain is fundamental to confirm it, using immunofixation. Characterization of the monoclonal component should not be considered complete without including IgD and IgE antisera, as the characterization may be limited to free light chains only. Given the rarity of cases, many laboratories lack in IgD and IgE antisera. Therefore, based on this experience, considering, and determining of IgD could prove helpful in further characterization of the monoclonal protein.

P45

FROM THROMBOCYTOPENIA TO ENDOCARDITIS. IMPORTANCE OF A PERIPHERAL BLOOD SMEAR ON CLINICAL ADVICE.

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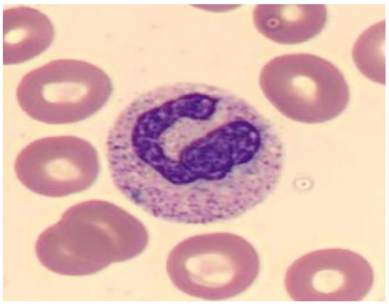
Introduction: Thrombocytopenia is generally defined as a reduction on the platelets count at the peripheral blood. Once we've ruled out the possibility of pseudothrombocytopenia induced by EDTA, we should have in mind situations such as hematologic neoplasia's, disseminated intravascular coagulation, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, sepsis, and others, as probable causes of new events of thrombocytopenia, making the observation of a peripheral blood smear (PBS) mandatory.

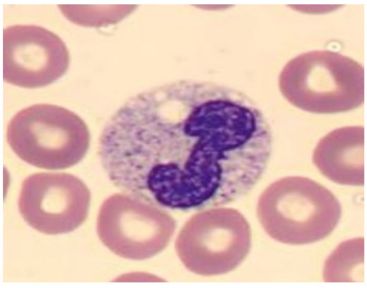
Clinical case: A 50-year-old male was admitted for nausea and generalized malaise one week after starting new antidepressant therapy. For start it was requested a simple laboratory evaluation with blood count, renal function, liver enzymes and ions, of which there were only changes in the blood count: discrete leucocytosis (14,39 G/L) and a severe thrombocytopenia (49 G/L). These changes led to a PBS, which confirmed the thrombocytopenia and revealed neutrophils

with toxic granulation, Dohle bodies and cytoplasm vacuoles. Therefore, it was decided to evaluate C-reactive protein and procalcitonin, obtaining levels of 37,53mg/L and 25,76ng/dL, respectively. With these results, we've informed the medical emergency department team and the patient was transferred to the intern medicine department for further investigation and care. Laboratory and clinical evaluation supported a diagnosis of sepsis due to meticilin-sensitive *Staphylococus aureus* infective endocarditis and a pneumonia with septic embolization pattern.

Discussion: In the presence of thrombocytopenia, the observation of a PBS helps us recognize some cellular morphologic changes useful in the identification of possible underlying causes. The recognition of Dohle bodies, neutrophil cytoplasmic hypergranulation and neutrophil cytoplasmic vacuolation is highly suggestive of and infectious disease, which must be investigated and reported to the clinician.

With this case, we want to reinforce that a medical attitude based on the correct clinical/laboratory information allows us to establish the best diagnostic approach and conduct and thus define the best therapeutic strategy for the patient.





P46

COLONIZATION WITH CARBAPENEMASE-PRODUCING ENTEROBACTERALES: SURVEILLANCE FROM 2016 TO 2023

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Background: The global emergence of multidrug-resistant Gram-negative bacteria, specially carbapenemase-producing *Enterobacterales* (CPE), is a significant public health problem due to the severity of the infections that they cause; in addition, treatment options are minimal, making clinical decisions difficult. This requires a screening strategy to limit their spread and guide the antibiotic therapy. Therefore, screening for colonization by CPE is imperative. Major risk factors associated with CPE are exposure to healthcare facilities, immunosuppression, overuse of broad-spectrum antibiotics, and the utilization of invasive devices.

Aim: Our study aimed to determine the colonization rate and the molecular profile of CPE in patients with risk factors admitted to our hospital.

Methods: A retrospective observational study was performed from January 2016 to December 2023, including all rectal swabs of patients with at least one risk factor. The cultural exam was carried out in the chromogenic medium chromID CARBA SMART (bioMérieux), which allows the detection of OXA-48 on one side of the plate and other CPE, mainly KPC and NDM on the other. The identification was made through the NG-Test CARBA 5 immunochromatographic test (NG-Biotech), which allows the identification of the five most prevalent carbapenemases families: KPC, NDM, IMP, VIM, and OXA-48. The final characterization is done through identification and antibiotic susceptibility testing using the Vitek-2 (bioMérieux) automated equipment. Data was collected and reviewed from clinical records.

Results: Upon admission, 187 patients (excluding duplicates) among the 3712 tested (13020 rectal swabs performed) had CPE, representing a colonization rate of 5.0%. The most prevalent *Enterobacterales* were *K.pneumoniae* (N=165, 88.2%), followed by *E.coli* (N=18, 9.6%) and *K.oxytoca* (N=4, 2.2%). KPC was the most frequent carbapenemase (49.2%), followed by OXA-48 (47.1%), NDM (2.7%), and VIM (1.0%). The colonization rates between 2016 and 2023 were 6.7%, 4.1%, 2.6%, 3.6%, 4.1%, 3.8%, 4.2%, and 10.7% respectively. The high value of 2023 was due to a *K.pneumoniae* OXA-48-like (OXA-181) outbreak.

Conclusions: Systematic and early screening is crucial in controlling CPE infections in hospitalized patients. It significantly contributes to controlling their spread and overcoming the therapeutic impasses.

P47

ASSESSMENT OF LABORATORY TURNAROUND TIME OF HIGH-SENSITIVITY CARDIAC TROPONIN T TESTING FROM THE EMERGENCY DEPARTMENT OF A LISBON HOSPITAL

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Background: Measurement of high-sensitivity cardiac troponin T (hs-cTnT) is pivotal in the diagnosis of acute myocardial infarction (AMI). [1] Prompt delivery of results from the laboratory is essential for effective management of AMI in emergency departments. International expert consensus recommends a turnaround time (TAT) for hs-cTnT within 60 min. [2]

Objectives: To characterise the laboratory TAT of hs-cTnT testing from our Hospital's Emergency Department during 2019-2023, based on the impact of SARS CoV-2 pandemic, automated validation using predefined criteria, equipment maintenance, and volume of routine work.

Methods: Serum hs-cTnT was measured using the ruthenium-based electrochemiluminescence immunoassay "Elecsys Troponin T hs" for Cobas platform (Roche Diagnostics). Data was retrieved from the laboratory information software Clinidata® XXI (n = 64439) and categorised by period (pre-pandemic: 2019; pandemic: 2020-2022; post-pandemic: 2023), mode of validation (automatic; manual), working day (equipment maintenance days; other days), or working hours (high work volume: 8:00am – 3:59pm; low work volume: 4:00pm – 7:59am). TAT was calculated as the time from specimen admission in the laboratory to result reporting. Statistical analysis was performed using GraphPad Prism (version 9.5.0), employing Tukey method for boxplots. The Mann-Whitney test was used to verify the significance of observed differences (two-tailed p value <0.05).

Results: Over the studied period, an average of 35 hs-cTnT tests were performed daily, with a mean laboratory TAT of 53 min (<30 min: 5 %; 30-45 min: 39 %; 45-60 min: 29 %; 60-75 min: 14 %; 75-90 min: 6 %; >90 min: 7 %). On average, it was spent 24 min for specimen's admission, 26 min for analysis, and 3 min for validation. The results found for each group are summarised in Table 1.

Table 1: Calculated hs-cTnT laboratory TAT for each studied group.

Group	Mean ± SD (min)	Median (min)	90 % percentile (min)	TAT ≤ 1h (%)
Period				
2019 (n = 13626)	50 ± 22	44	75	78
2020-2022 (n = 39516)	55 ± 26	49	84	70
2023 (n = 11297)	50 ± 23	44	76	77
Mode of validation				
Automatic (n = 42332)	52 ± 24	46	78	75
Manual (n = 22107)	56 ± 27	49	86	69
Equipment maintenance				
Maintenance (n = 23315)	56 ± 26	49	85	70
Non-maintenance (n = 41124)	52 ± 24	46	78	75
Routine work volume				
Low (n = 41565)	48 ± 22	43	70	83
High (n = 22874)	62 ± 27	57	95	56
Overall				
All analyses (n = 64439)	53 ± 25	47	81	73
Ideal conditions* (n = 6991)	44 ± 20	40	62	88

**Non-pandemic period (2019 and 2023), with automatic validation, without maintenance days, during low routine work volume (4:00 pm – 7:59 am). SD = standard deviation; TAT = turnaround time.*

Conclusions: Our laboratory fulfilled the international recommendation of a hs-cTnT TAT <60 min in 73 % of the cases, during 2019-2023. Outliers (TAT >96 min, 5 %) were mainly due to late additional requests after specimen admission in the laboratory or delayed transport after registration. Prolonged admission process, SARS-CoV-2 pandemic, manual validation, equipment maintenance, and high routine work volume contributed to delayed results (9 min; 15 % less of reports on time). Identification of these factors allows further measures to enhance laboratory response.

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P48

"THERE'S BEEN A CYBERATTACK… WHAT NOW?" – A NIGHTMARE BASED ON A TRUE STORY.

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Introduction: On the night of the 25th to the 26th of April 2022, a Group II hospital in the Lisbon Metropolitan Area suffered a cyberattack that disrupted its main servers, rendering all software applications inoperable, restricting internet access and the use of all computers. This included the Laboratory Information System (LIS) and all lab software. The Clinical Pathology Service of said hospital, processes different test samples ranging from haematology, coagulation, flux cytometry, clinical chemistry, immunology, molecular biology, and microbiology; in an average daily sample load of approximately 8000 sample tests.

Case report: Following the shutdown of the main hospital servers, the lab implemented its emergency contingency plan, delineated in its internal standard operating protocols, which specified: the use of paper test request forms; at sample reception, the use of a sticker (created in-house) with the patient institution process number and date, and a copy of the paper form for each different lab section; Tests had to be programmed on a one-by-one basis on the lab analysers; After processing, a copy of the test results taken directly from the analysers was stapled to the paper form and manually validated by a Clinical Pathologist; Distribution through a drawer system, with each one attributed to a specific department, collection of which was handled by said departments staff; Work-flow and organizational restructure, with increase in work hours. Outpatient sample collection was limited to specific populations, later being handled by an external lab. This continued until partial restoration of lab services in May 2022.

Results: Despite best efforts, turn-around-time for reports got compromised. The use of only patients' institution code and date, all the while archiving all paper request forms, meant traceability was severely compromised. These reports were collected by the patients' attendants from the lab itself. Many reports were not claimed. The data generated during this one-month period could not be stored electronically.

Conclusion: In a digital era, any institution might be a target for a major cyberattack. It is imperative that strong, strict cybersecurity protocols and robust standard operating protocols be established, so that personnel are prepared to handle such a crisis.

P49

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) COLONIZATION AND RISK FOR MRSA BACTEREMIA: 8 YEARS EVALUATION

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Background: MRSA is a common hospital-acquired pathogen that can cause a broad spectrum of infections, including bacteremia, which is associated with an unfavorable outcome, significant morbidity, and mortality. MRSA colonization has been considered a risk factor for the development of infection, particularly bacteremia, with central venous catheters and pneumonia being the most common sources of infection. To prevent these infections, programs to prevent and control the transmission of MRSA have been implemented, including screening and decolonization of carriers with nasal mupirocin and chlorhexidine bathing.

Aim: This study aimed to evaluate the risk of MRSA bacteremia in individuals colonized with MRSA in our institution.

Methods: A retrospective observational cohort study was performed from January 2016 to December 2023 (8 years), including all nasal swabs of admitted patients with risk factors for MRSA infection and all MRSA-positive blood cultures performed in our hospital. Polymerase chain reaction Cepheid Xpert MRSA NxG test was performed in all nasal swab patients. Patients were considered colonized if a nasal swab tested positive for MRSA at the same time or > 24 hours before the venipuncture, which led to the diagnosis of bloodstream infection. The statistical analysis was carried out using Excel.

Results: The study showed that out of 3757 patients (excluding duplicates) who underwent screening for nasal MRSA colonization, 537 (14.3%) were colonized with MRSA. Between 2016 and 2023, colonization rates decreased by 50%, from 22% in 2016 to 11% in 2023. During this period, we detected 121 patients with *Staphylococcus aureus* bacteremia, 46 of which were due to MRSA (prevalence rate of 38%). There were 28 cases of MRSA bacteremia among colonized patients, with a prevalence of 5.2%. Eighteen cases of MRSA bacteremia occurred in non-MRSA-colonized patients, with a prevalence of 0.56%. The odds of developing MRSA bacteremia for patients who were nasally colonized with MRSA compared with those who were not colonized were 9.8.

Conclusions: This study emphasizes the significance of actively screening patients for MRSA colonization and performing decolonization if the results are positive, especially in a hospital setting. In our institution, the risk of MRSA bacteremia is 9.8 times higher among colonized patients than non-colonized ones.

P50

DEFINING PATIENT SAFETY WHEN EXCHANGING FROM IMMAGE TO OPTILITE ANALYSER FOR CERULOPLASMIN AND HAPTOGLOBIN DETERMINATIONS

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Introduction: Immage analyzer uses the gold standard method of nephelometry, to determine Ceruloplasmin (CP) and Haptoglobin (HP).

Optilite uses an immunoturbidimetric method to determine CP and HP.

Objective: To evaluate the patient biological safety for the diagnostic on our population and for the monitorization of individuals, on the moment we exchange from nephelometry to the Optilite immunoturbidimetric method.

Material and Methods: We used 15 patient samples to compare the results on Optilite and Immage for CP and 13 patients for HP.

We used the linear regression and percent differences comparison on an Excell Sheet.

We compared the obtained results with the known between-subject biological variation (CVg) for CP and HP, to ascertain the diagnostic safety for our population, and the known intraindividual-subject biological variation (CVi) to ascertain the safety for the monitorization of individuals.

Results: On the linear regression we observed a correlation of R^2=0,94 for CP and R^2=0,99 for HP, and the percent differences average of 13% for CP and 8,1% for HP.

The European Biological Variation Database (EFLMBV) (1), reveals that populational CVg for CP is 15,1% , and 39.0% for HP. It also determined that intraindividual CVi for CP is 5.0%, and 8.6% for HP.

Conclusion: The results of the analytical comparison shows good and very good concordance between both methods, for CP and HP determination, respectively.

The average differences of results for both CP and HP, are below the respective CVg, that confirms this imunoturbidimetric method is as safe for the diagnostic of our population, as nephelometry.

For the HP determination, the average difference between both methods is below CVi, that shows to be also safe for the monitorization of individuals, when using both methods data for the same patient.

References

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P51

EVALUATION OF A SERUM POOL AS AN INTERNAL QUALITY CONTROL SAMPLE IN THE AREA OF CLINICAL CHEMISTRY

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[2]*Department of Epidemiology External Quality Assessment, National Institute of Health Doctor Ricardo Jorge, Lisbon, Portugal*
[3]*Faculty of Pharmacy of the University of Lisbon, Lisbon, Portugal*

Introduction: This work was carried out as part of *ProMeQuaLab* (Laboratory Quality Improvement Project for Portuguese-speaking Countries), which began in 2015 under the coordination of INSA, IP and aims to improve the quality of laboratory results.

Internal quality control (IQC) is used to monitor analytical performance and validate patient results.

Objective: To evaluate a pool of sera to use as IQC sample in clinical chemistry (CQ) for glucose, urea, creatinine, total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and iron.

Materials and Methods: Preparation of a pool of sera (6 volunteers). Freeze-dried commercial control samples (CC), two concentration levels. The parameters were analysed on the Cobas Integra®400 plus Roche Diagnostics.

An Excel spreadsheet was used to calculate the mean, standard deviation (SD) and Coefficient of Variation (CV%). The CV% of the pool and CC were confronted with the analytical performance specifications (APS) based on biological variability – EFLM 2023.

Results: Samples from a pool of sera were evaluated in parallel with the IQC CC (Level 1 and 2) in the analytical runs from April to August 2023.

The CV% obtained for the parameters glucose, TC met the desirable APS in the pool (2.1, 1.5, respectively) and in the CC, Level 1 (1.5, 1.8, respectively) and Level 2 (2.4, 1.5, respectively).

The CV% of urea and iron met the desirable APS in the pool (4.7, 6.9, respectively) and the optimal ones in the CC, Level 1 (2.9, 4.6, respectively) and Level 2 (3.4, 3.6, respectively).

The AST CV% met the minimum APS for the pool (6.1) and the optimal APS for the CC Level 1 (2.0) and Level 2 (1.6). The ALT CV% met the minimum APS for the pool (7.5, up to the 22nd day) and the desirable ones for CC Level 1 (3.3) and Level 2 (2.5).

The CV% of creatinine did not meet the minimum APS for the pool, which may be partly explained by the lower range of values in the pool (mean =0.71 mg/dL) than in the CC.

Conclusion: The CC generally have lower CV%, but the pool showed the desirable or minimum APS of the EFLM for the analytical parameters analysed, except creatinine, and presented some benefits:

a) Introduction of concentrations close to the clinical decision level, with good reproducibility results (iron, glucose and TC).

b) Commutability, low cost and accessibility.

The pool has proven to be a viable and affordable alternative for IQC, especially when the availability/cost of CC is a significant challenge.

P52

SHEDDING LIGHT ON PERSISTENT POLYCLONAL B-CELL LYMPHOCYTOSIS: A CASE REPORT AND CLINICAL INSIGHT

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Persistent Polyclonal B Cell Lymphocytosis (PPBL) is a rare disorder predominantly diagnosed in middle-aged women who smoke. It is characterized by moderate, chronic, and absolute lymphocytosis, the presence of binucleated lymphocytes in peripheral blood examination, and moderate elevation of serum IgM. The immunophenotype of the B cells expanded is CD27+IgM+IgD+. The prevalence is unknown because most patients are asymptomatic. The clinical course of PPBL is typically benign, but it's uncertain whether it may lead to the emergence of a malignant proliferative disorder.

We report the clinical case of a 41-year-old Caucasian woman referred to the hematology consultation for persistent leukocytosis. An abdominal-pelvic ultrasound and computed tomography scan were previously performed, which revealed no abnormalities except for a liver nodule with no suspicious changes and an ovarian cyst. She was a smoker (24 pack-years). Upon evaluation in the consultation, she was asymptomatic, and the physical examination was normal. Laboratory analysis revealed absolute lymphocytosis of 11.25 x 10^9/L, with no anemia or thrombocytopenia, and no other significant alterations. Peripheral blood smear described the presence of some binucleated lymphocytes. Immunoglobulin levels (IgG, IgA, and IgM) were within normal ranges. Serum protein electrophoresis was unremarkable. Flow cytometry revealed an increase in B lymphocytes (19.1%). The B-cell population was polyclonal, with 88% expressing CD27+. These findings helped rule out the suspicion of a lymphoproliferative hematologic disorder. The patient was discharged from the hematology consultation and advised to maintain her regular clinical and analytical follow-up with her primary care physician.

It is essential to be aware of the existence of this entity to recognize and distinguish it from other hematologic disorders, thereby avoiding submitting the patients to unnecessary invasive diagnostic or therapeutic procedures. Further research is needed to elucidate the underlying mechanisms of PPBL and its potential connection to malignant proliferative disorders.

P53

ISOLATION OF ACTINOTIGNUM SCHAALII IN URINE CULTURE

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Introduction: Actinotignum schaalii is a facultative anaerobic, coccobacillus Gram positive, urease and oxidase negative. Colonies are small <1mm, gray, alpha-hemolysis or gamma-hemolysis. These characteristics mean that it can go unnoticed

during growth in the culture, due to the invasion of other bacteria. It belongs to the flora of the genitourinary tract, however it may be responsible for urinary infections in the elderly, immunocompromised children or with urinary system pathology. There are only fews isolations already described, cases of urinary infections, bacteremia, abscesses and cellulitis.

Clinical case: Female, 45-year-old, went to the hospital complaining of hematuria, pollakiuria and suprapubic pain. With a history of iron deficiency anemia, cervical cancer, bilateral ureteral stenosis, urethral stent and with urinary incontinence. Analytically: Hb 10.9mg/dL, leukocytes 3,700x109/L, C-reactive protein 22.5mg/dL. Urine culture was ordered and revealed rare epithelial cells, many leukocytes, many erythrocytes. Actinotignum schaalii was isolate >100,000 cfu/ml, being sensitive to ampicillin, amoxicillin/ac. Clavulanic, cefuroxime and resistant to ciprofloxacin. She was treated with cefuroxime and had clinical improvement.

Discussion: This microorganism was recently identified and it is difficult to isolate. Most urine isolations are associated with elderly patients and/or those with urological conditions. This patient has multiple pathologies of the urinary system that are risk factors and make this microorganism the cause of the urinary infection symptoms presented.

When isolated in urine culture, Actinotignum schaalii should be considered a causative agent of urinary infection in some patients.

Declaration of conflict of interests: The authors declare that they have no conflicts of interest.

P55

COMPARISON OF MYCOPLASMA IST 3 AND ALLPLEX STI ESSENTIAL ASSAY FOR THE DETECTION OF MYCOPLASMA HOMINIS AND UREAPLASMA SPP. IN GENITAL SWABS

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Introduction: Mycoplasma hominis and Ureaplasma spp. are colonizing agents of the male and female urogenital tract. Asymptomatic carriage of these agents is common and its association with Sexually Transmitted Infection syndromes and complications can be questioned. Nevertheless, these agents are routinely tested in Microbiology Labs as proposed agents of urethritis in male patients and cervicitis, pelvic inflammatory disease, endometritis and infertility in female patients.

The aim of the study was to evaluate the agreement of a newly implemented culture based method that allows identification, quantification and antibiotic susceptibility testing for M. hominis and Ureaplasma spp. with the Polymerase Chain Reaction (PCR) multiplex assay used in the routine.

Methods: A retrospective study was conducted and results obtained by gallery culture (Mycoplasma IST 3, BioMérieux®) were compared with the ones found by PCR (Allplex™ STI Essential Assay Q (MH,UU), Seegene®). Samples tested by both methodologies over a period of 6 months were selected.

Results: A total of 90 vaginal swab samples from women were tested by both methods. Results obtained by culture had an overall percent agreement with PCR testing of 91% for Ureaplasma spp. and 95,5% for M. hominis. Positive Percent Agreement for the detection of these agents using Mycoplasma IST 3 was 89,6% for Ureaplasma spp. and 66,7% for M. hominis. Its Negative Percent Agreement was 92,7% for Ureaplasma spp. and 100% for M. hominis.

Discussion: Mycoplasma IST 3 showed overall good concordance with PCR testing for the three studied agents. The culture based method has the advantage of allowing susceptibility testing. The excellent Negative Percent Agreement found in this work suggests an algorithmic approach can be implemented in which a culture method is done only after a positive result is obtained by PCR.

P56

CASE REPORT: AUTOIMMUNE HEMOLYTIC ANEMIA

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Introduction: Autoimmune hemolytic anemia (AIHA) can be triggered by genetic mutations, environmental factors (such as drugs and infections), lymphoproliferative disorders, and autoimmune diseases, among others. Nevertheless, over half of AIHA cases are idiopathic.

Case Report: A 52-year-old woman was referred to a hemato-oncology consultation in March 2023 for anemia, initially normocytic and normochromic, which progressed to macrocytic. Objective examination revealed icteric sclerae, cushingoid facies, and mucocutaneous pallor. Medical history of SARS-CoV-2 infection in February 2021, which caused asthenia and decreased muscle strength in both upper and lower limbs. No reported regular medication use or drug allergies. In October 2023, laboratory results show anemia (hemoglobin 7.4 g/dL), high reticulocyte count (24.91 %), and biochemical parameters associated with hemolysis (undetectable haptoglobin, total bilirubin 1.13 mg/dL, lactate dehydrogenase 529 U/mL). Perip heral blood smear, showed a reduced erythrocyte count, anisochromia, hypochromia, polychromasia, basophilic stippling, spherocytes, and slight erythrocyte agglutination. The direct antiglobulin test, considered the gold standard confirmatory test for AIHA, was positive, with monospecificity for IgG. Abdominal ultrasound, fluorescence *in situ* hybridization, immunophenotyping of bone marrow aspirate, and serology (testing for human immunodeficiency virus, hepatitis C virus, hepatitis B virus, and syphilis) yielded no significant findings. Treatment progressed to third-line therapy with azathioprine, after lack of therapeutic response with prednisolone and rituximab.

Discussion: This case report highlights the complex etiology associated with AIHA. Viral, bacterial, and protozoal infections have been linked to this type of anemia, with cases of AIHA following SARS-CoV-2 infection documented. In this case report, since no other cause could explain the clinical presentation, it is considered that the prior SARS-CoV-2 infection might be the origin. AIHA might represent a late manifestation or have been covert by the marked musculoskeletal symptoms. The pathophysiological interaction between AIHA and SARS-CoV-2 infection demands further investigation to clarify the temporal emergence of both conditions and the relationship between the severity of COVID-19 and this hematological disorder.

***Figure* 1** - Peripheral blood smear showing anisochromia, hypochromia, polychromasia, basophilic stippling, spherocytes and slight erythrocyte agglutination.

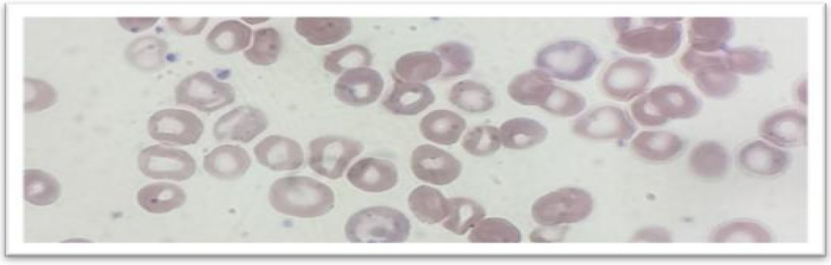
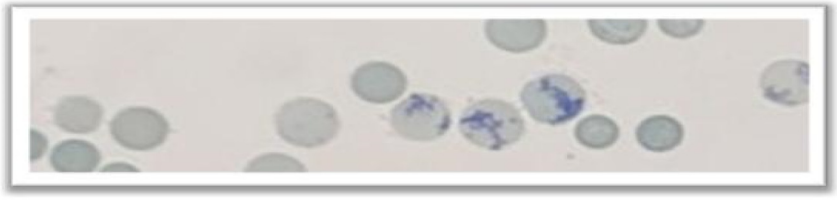


***Figure* 2** - High reticulocyte count, evidenced by staining with Brilliant Cresyl Blue solution.



P57

HEPATITIS DELTA - A REVIEW

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Hepatitis Delta Virus (HDV) is a defective and small hepatotropic RNA virus reliant on the Hepatitis B Virus (HBV) surface antigen (HBsAg) for its life cycle, posing a global health threat. HDV infection may occur by HBV-HDV co-infection or superinfection, and the latter is usually more severe. HDV infection leads to the most severe form of viral hepatitis, associated with increased risks of cirrhosis and hepatocellular carcinoma. HDV transmission occurs via parental or mucosal contact with infected blood or body fluids. Epidemiological data on HDV remains inconsistent despite its advancements, and therefore requires further accurate diagnosis and public health strategies[MJL(1].

WHO estimates global HDV prevalence at 15-20 million people, 5% to7% of chronic HBV carriers. High endemicity regions include Central Africa, South America, and the Mediterranean, with eight HDV genotypes globally. Genotype distribution varies, reflecting diverse epidemiological landscapes[MJL(2] .

HDV infection diagnosis relies on high levels of anti-HDV IgG and IgM, and HDV RNA detection in serum, especially crucial in HBsAg-positive patients. European guidelines recommend HDV screening in all HBV-infected individuals.

HBV nucleos(t)ide analogues, such as entecavir or tenofovir, are ineffective against HDV. The entry inhibitor, bulevirtide, was recently approved in Europe, whereas Pegylated interferon alfa is the only treatment available in most countries.

Conclusion: Only approximately 20% to 50% of people infected by hepatitis D have been diagnosed. This is due to a lack of awareness and limited access to reliable diagnostic tests for the HDV antibody and HDV RNA. The HBV vaccine prevents HDV infection by preventing HBV infection, but no vaccines are available to protect those with established HBV infection against HDV. HDV management poses unique challenges, with chronic HDV (CHD) infection being severe. PegIFNa remains the primary treatment of choice while emerging therapies offer hope for improved outcomes.

Underestimation of HDV prevalence emphasizes the need for a systematic screening as well as proactive interventions to help mitigate the global burden caused by this disease. Despite recent advancements, HDV continues to pose significant health risks, which demands the implementation of comprehensive strategies to effectively tackle this neglected disease.

[MJL(1]https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4208707/
[MJL(2]https://pubmed.ncbi.nlm.nih.gov/28903779/

P58

ASSESSMENT OF THE HEAVY/LIGHT CHAIN ASSAY IN A POPULATION WITH DIFFERENT MONOCLONAL GAMMOPATHIES

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Heavy/Light Chain (HLC) assay separately quantifies specific pairs of heavy/light chains of each of immunoglobulin (Ig) isotypes (IgGk/IgGλ, IgAk/IgAλ, IgMk/IgMλ) and is available in laboratories to manage Monoclonal Gammopathies (MG) patients. HLC ratio identifies monoclonality, while measuring the increased expression of the monoclonal Ig (iHLC) separately from the polyclonal Ig of the same isotype (uHLC). International guidelines recommend using HLC when the Monoclonal (M)-protein overlaps other serum proteins in proteinogram (EPS) and data shows that suppression of uHLC as a prognostic value in MG patients.

Identify the importance of HLC quantification in diagnosis and prognosis of MG.

We studied 52 samples from 46 patients: 39 MG of Undetermined Significance (MGUS), 6 Multiple Myeloma (MM) and 1 Waldenstrom's Macroglobulinemia (WM). Hevyliteâ (The Binding Site Group Ltd.) were used for HLC isotype quantification. Serum protein electrophoresis (SPEP) and serum immunotyping were used to quantify and characterize M-protein by capillary electrophoresis (Capillarys 3-Sebia). Total IgA, IgG and IgM were determined by turbidimetry (Roche Diagnostics). Comparison were analized by Passing–Bablok regression.

29 samples had an abnormal HLC rt, identifying clonality. 20 samples had a normal rt, 16 from MGUS, (3 patients had an increase iHLC) and 4 samples were from MM after treatment. All MGUS IgAk had an abnormal HLC rt, median 19,86 (RR: 0.91-2.4), all but one presented with a mild elevation of M peak in the β-2 region of SPEP, making it difficult the quantification of the M protein. Median values of M protein in SPEP were 4.96 g/L (1.92-34.68g/L) and iHLC was 7.50 g/L (1.49-51.94g/L). Correlation between both methods was $R^2 = 0.7639$. Our results show that more patients present uHLC immunosuppression (uHLC below RR), than suppression of polyclonal immunoglobulins, 18.9% vs 5.4%. A good correlation was found comparing total Igs versus the sum of 2 pairs of the same isotypes by HLC.

When M-peak has little expression HLC rt can show a monoclonal process occurring, more evident in our MGUS IgA patients. HLC rt normalization in patients with MM, undergoing treatment, may indicate disease remission. uHLC immunosuppression emerges earlier in more patients than Igs immunoparesis, becoming a prognostic biomarker. Follow-up of these patients over time and the evaluation of HLC, seems promising, introducing new information not available through traditional tests.

P59

EVALUATION OF SERUM FREE LIGHT CHAINS AS AN ENHANCED MONITORING FOR LIGHT CHAIN MULTIPLE MYELOMA

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Light chain multiple myeloma (LCMM) constitutes, approximately, 15% of patients with multiple myeloma (MM). This type of MM is characterized by the overproduction of only light chain. LCMM has a more aggressive course and poorer prognosis when compared with others MM types.

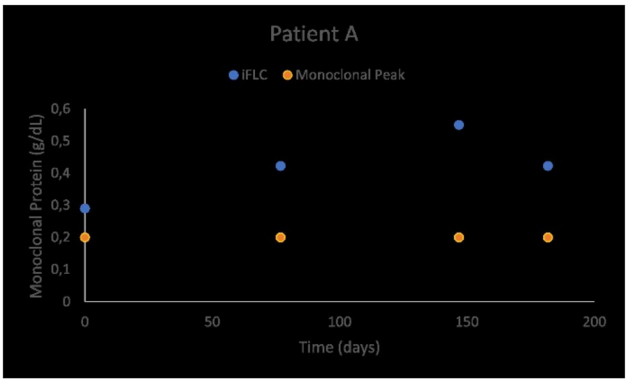
Serum protein electrophoresis (SPEP) and quantification of serum free light chains (sFLC) are important to detect and monitor monoclonal proteins in monoclonal gammopathies, like multiple myeloma. Frequently, in LCMM the SPEP show no M-spike because of the absence of complete immunoglobulins (Igs) secretion by malignant plasma cells.

The aim of this study is to evaluate the best method to follow-up the monoclonal protein in LCMM.

All LCMM patients to whom concentrations of sFLC or M-peak by SPEP were determined at the institution, between June 2023 and January 2024, were reviewed. All samples were analysed by SPEP using the Sebia Minicap (Sebia USA, Norcross, GA, USA), sFLC were determined with Freelite® kits on Optilite analyser (The Binding Site Portugal Specialist Protein Company) and immunoglobulins were quantified in Cobas 8000® analyser (Roche Diagnostics International Ldt.).

During this time, twenty-seven LCMM patients (13 λ LCMM and 14 κ LCMM) in a total of 101 samples, were identified in the Clinical Pathology Department of our hospital center. In our cohort, 14 of 101 samples had a quantifiable M-peak with concentrations ranging from 0.1 to 0.4 g/dL. Such concentrations make this method unsuitable for LCMM monitoring since the International Myeloma World Group guidelines for measurable disease in serum is defined by M-Peak >1.0 g/dL. Regarding sFLC, which criteria for measurable disease are sFLC >10 mg/dL, an abnormal FLC ratio was observed in all 14 samples and sFLC concentrations were higher (between 152.41 and 2901.62 mg/dL). Nine of these samples also presents a suppression of polyclonal Igs, a common feature in LCMM.

When we compare the sFLC concentration and quantification of M-spike, we observed the FLC is variable, but the component of M-spike remains over time. Such differences have an important role in patient follow-up, so we can conclude quantification of sFLC is the most sensitive and precise technique for monitoring LCMM patients.



P60

IT'S TRIPLETS! BABY, LISTERIA MONOCYTOGENES AND… SOMETHING ELSE

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Introduction: *Listeria monocytogenes* is a significant pathogenic bacterium during pregnancy. While it often remains asymptomatic in pregnant women (or presents as a flu-like syndrome), it can lead to serious complications for the fetus. *L. monocytogenes* is a Gram-positive, rod-shaped, facultative anaerobic, β-hemolytic and non-spore-forming bacterium. Its diagnosis can be challenging because, as a Gram-positive bacterium, it may be mistakenly considered a skin contaminant.

Case Description: A 23-year-old pregnant woman at 24 weeks' gestation, initially monitored in São Tomé and Príncipe, presented to the Emergency Department with epigastric pain. She was afebrile with no significant findings on physical examination. Analytically, she exhibited hepatic cytolysis and iron deficiency anemia. Viral serologies and abdominal ultrasound were unremarkable.

Three sets of blood cultures were collected, turning positive after 18 hours of incubation (BACTEC™). Gram stain revealed the presence of Gram-positive rods. Colonies on chocolate blood agar plates, after 24 hours, were small and grayish. Identification with VITEK®2 (ANC) revealed *Lactobacillus plantarum* and identification with VITEK®2 (GP) revealed *L. monocytogenes*. In light of the pregnancy context and the observation of colonies exhibiting β-hemolysis on blood agar plates, the result was reported as *L. monocytogenes*. The patient was hospitalized and initiated treatment with ampicillin.

During hospitalization, she maintained symptoms and experienced vomiting with the expulsion of a 15 cm worm (figure 1). Subsequently, a stool parasitological examination was performed, revealing the presence of *Ascaris lumbricoides* eggs (figure 2). The patient was treated with albendazole and had a favorable outcome.

Discussion: The presence of Gram-positive bacteria in blood cultures from a pregnant woman should raise suspicion of *Listeria spp*. Early diagnosis and treatment are crucial to prevent complications for both the pregnant woman and the fetus.

However, an established diagnosis of listeriosis does not rule out the presence of other disease-causing agents, particularly considering the immunological status inherent to pregnancy. Factors such as dietary and hygiene habits and travel history can provide essential clues for the presence of other infectious agents.

Figura 1. *Ascaris lumbricoides* - verme adulto

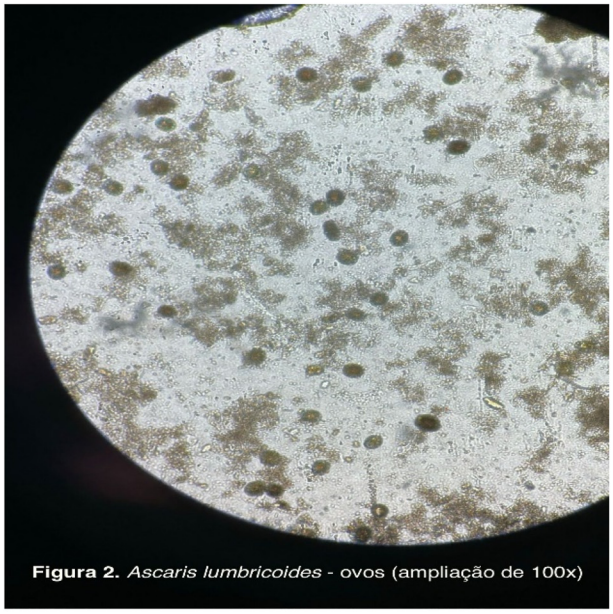

Figura 2. *Ascaris lumbricoides* - ovos (ampliação de 100x)

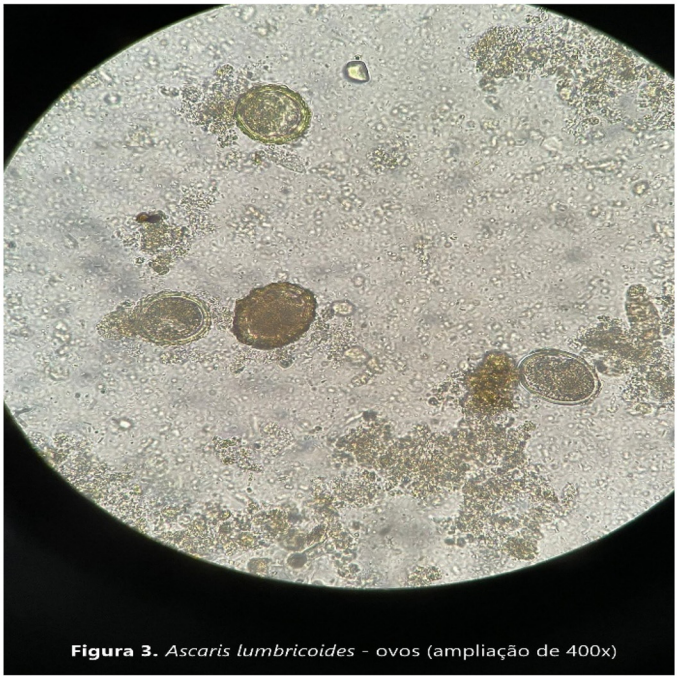

Figura 3. *Ascaris lumbricoides* - ovos (ampliação de 400x)

P61

QUANTIFICATION OF UNCERTAINTY ASSOCIATED WITH THE PRE-ANALYTICAL PHASE: HEMOSTASIS STUDIES

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Introduction: ISO15189 establishes uncertainty of the result as mandatory requirement in Clinical Laboratories Accreditation process. Uncertainty may arise from any of the phases of the process. With around 70% of laboratory errors occurring in pre-analytical phase, this stage relates directly to uncertainty, as human intervention and inadequate execution of procedures represent sources of variability. The assessment of pre-analytical uncertainty is fundamental to determine how these sources can affect the final result. Coagulation tests are amongst the most susceptible, due to poor standardization of processes such as homogenization, transport and storage.

Objective: In collaboration with SYNLABHealth Algarve, Accredited by ISO15189, the study analyzed the consequence of pre-analytical variability in hemostasis (PT, aPTT and fibrinogen). It aimed to determine combined (u_c) and expanded (U) uncertainty, with focus on homogenization, transport and storage.

Methods: Two parallel venipunctures were performed (43 volunteers), to compare the standard with the experimental protocol. The data pairs were evaluated and converted into total coefficient of variation (*CV*) values. Standard pre-analytical uncertainty for each variable was obtained by subtracting the analytical *CV* from the total *CV*. Combined uncertainty (u_c) was determined considering the standard uncertainty for each variable.

Results: Freezing was the source of variability with higher associated uncertainty, showing more relevance on the fibrinogen test. The absence of homogenization had greater impact compared to vigorous mixing. In transport, fibrinogen was the most affected test. The puncture was the factor with least potential variation to the results. Considering a practical case, respectively for fibrinogen, aPTT and PT, the U found was 2.4%; 6.6% and 16.6%.

Conclusion: The study provided knowledge about the influence of pre-analytical sources of variability, quantifying its impact in the clinical results. Pre-analytical factors affecting results should be considered in laboratory medicine and this study demonstrated the relevance of including this estimative in laboratory accreditation processes. Estimating pre-analytical uncertainty should be emphasized and integrated in the ISO15189 measurement uncertainty requirement. In the future, competent entities, should indicate clear methodologies to uncertainty determination and validate reference tables to compare uncertainty values.

P62

TRANSIENT MYELOPROLIFERATIVE SYNDROME: A CASE REPORT

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Clinical Case: A male newborn, 24 days old, born at term (37 weeks), with appropriate weight for gestational age (2910g) and Apgar scores of 8/9/10. The infant has Down syndrome (DS). Admitted to the Pediatric Emergency Department due to respiratory distress, hypotonia and hypoxemia. Physical examination revealed no hepatomegaly or splenomegaly. Chest X-ray showed no abnormalities. Echocardiogram revealed persistent left superior vena cava (PLSVC) draining into a dilated coronary sinus. Laboratory analysis showed hemoglobin 12.5g/dl (normal range: 14.0-18.0 g/dl), white blood cells $14.9x10^3$/µl (normal range: $5\text{-}20x10^3$/µl), neutrophils $4.2x10^3$/µl (normal range: $0.8\text{-}9x10^3$/µl), lymphocytes $7.5x10^3$/µl (normal range: $2\text{-}14x10^3$/µl), metamyelocytes 1%, myelocytes 1%, blasts 10%, and platelets $131x10^3$/µl (normal range: $140\text{-}440x10^3$/µl). Other biochemical parameters were within normal limits, except for total bilirubin 2.88 mg/dl (normal range: 0.20-1.20 mg/dl), direct bilirubin 0.74 mg/dl (normal range: 0.050-0.30 mg/dl) and indirect bilirubin 2.14 (normal range: 0.00-0.60 mg/dl). Peripheral blood immunophenotyping showed 20.5% blasts with CD34+, CD117+, CD45+, CD13-, CD11-, CD19-, CD3- and HLA-DR-. After 12 days of hospitalization, improvement in respiratory symptoms and no need for oxygen therapy for 3 days, the patient is discharged. Referred to Pediatric Oncology for genetic study and to Pediatric Cardiology for PLSVC monitoring.

Case Discussion: The clinical presentation of respiratory distress, hypotonia and hypoxemia led to the hospitalization of a male newborn with DS. During hospitalization, cardiovascular alterations, jaundice and TMS were observed. This case represents a typical presentation of TMS in individuals with DS. The condition is detected in the neonatal period by the

presence of a left shift in the granulocytic series, blast presence and thrombocytopenia. Most newborns with TMS experience spontaneous remission and do not require pharmacological treatment. However, this patient needs monitoring due to a 20% risk of developing acute leukemia following TMS remission. The most common type of AML-DS is M7 (megakaryoblastic), followed by M4 (myelomonocytic) and M0 (undifferentiated). In cases where therapy fails, the mortality rate for AML-DS is high, requiring the exploration of new therapeutic alternatives.

P63

BEYOND RELAPSING POLYCHONDRITIS AND MACROCYTIC ANAEMIA – A COMPLEX DIAGNOSIS

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Introduction: VEXAS syndrome is a rare somatic monosomic disorder caused by a mutation in the UBA1 gene, manifesting with haematologic and autoinflammatory symptoms, primarily in males aged over 50. The definitive diagnosis entails genetic testing targeting the UBA1 gene.

Case report: A 74-year-old man presented with an 8-month history of superficial venous thrombosis, low back pain displaying an inflammatory pattern, and anorexia. Laboratory evaluation revealed macrocytic anaemia (haemoglobin concentration [Hb] of 8,8 g/dL, mean corpuscular volume [MCV] of 102 fL) alongside elevated inflammatory markers (C-reactive protein serum concentration [CRP] of 4.27 mg/dL, erythrocyte sedimentation rate [ESR] of 120 mm/h), without evidence of vitamin deficiencies. Magnetic resonance imaging showed asymmetrical bilateral sacroiliitis. Subsequent referral to Rheumatology and Haematology led to initiation of prednisolone therapy at 15 mg/day, yielding clinical improvement within a month. However, after three months, the patient reported a 10-day duration of recurrent evening fevers and was admitted to the Infectious Diseases unit. Laboratory assessment revealed persistent macrocytic anaemia (Hb of 8.5 g/dL, MCV of 103.1 fL) and exacerbated inflammatory markers (CRP of 15.0 mg/dL, ESR of 88 mm/h) alongside diminished glycosylated ferritin levels (15%). Due to recurrence of auricular chondritis, and a persistent febrile state, a presumptive diagnosis of relapsing polychondritis was made, prompting reintroduction of prednisolone at 20 mg/day and subsequent clinical improvement.

A bone marrow aspirate and osteomedullary biopsy were undertaken, revealing no evidence of dysplasia but extensive vacuolization in erythroid and myeloid precursors. Genetic testing unveiled a pathological variant in the UBA1 gene.

Discussion: VEXAS syndrome, a newly identified condition with an estimated prevalence of 1:14,000, stems from a mutation in the UBA1 gene, resulting in the production of a truncated protein with diminished activity. Diagnosis is complex due to its diverse symptomatology, but the presence of macrocytic anaemia and vacuolization of myeloid and erythroid precursors is observed in 100% of affected individuals, which, combined with autoinflammatory symptoms, should prompt mutation screening.

P64

CHALLENGES IN DIAGNOSING CENTRAL NERVOUS SYSTEM INVOLVEMENT BY DIFFUSE LARGE B CELL LYMPHOMA – A CASE REPORT

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is a high-grade non-Hodgkin lymphoma known for its aggressive growth and heterogeneity. The majority of patients with DLBCL present with nodal disease. Secondary involvement of the

central nervous system (CNS) by DLBCL is uncommon, occurring in approximately 3.5% to 5% of cases, particularly in some high-risk clinical settings.

Case report: A 47-year-old woman, HIV-1 positive and recently diagnosed with stage IIIA DLBCL, not otherwise specified (NOS), with isolated MYC rearrangements, presented with a 7-day history of severe holocranial headache, nausea, vomiting and altered gait. Altered behaviour, visual hallucinations and photophobia were also reported. On examination, she was disoriented in time and space and exhibited hyperfamiliarity and moderate dysarthria and hypophonia. Additionally, her gait was broad-based and markedly unstable, necessitating bilateral support. Cerebrospinal fluid (CSF) analysis revealed elevated protein levels (625 mg/dL) and severe hypoglycorrhachia (2 mg/dL), with 368 cells/μL, predominantly polymorphonuclear, using a Nageotte chamber. Empirical antibiotic therapy was initiated due to suspected bacterial meningoencephalitis. Subsequent microscopic examination of a May-Grünwald-Giemsa-stained cytocentrifuge preparation revealed predominantly large lymphoid cells with maintained nucleus/cytoplasm ratio. The cytoplasm appeared hyperbasophilic, occasionally showing slight vacuolization, and the nuclear contours were irregular/cleaved with semicondensed chromatin, sometimes displaying evident nucleoli. Flow cytometry confirmed CNS infiltration by DLBCL cells. Furthermore, a negative result from a CSF molecular multiplex test supported the absence of infectious agents associated with meningitis or encephalitis.

Discussion: The diagnostic challenges in this case underscore the importance of maintaining a high index of suspicion when encountering neurological symptoms in patients with DLBCL, NOS. Distinguishing between infectious and neoplastic aetiologies is crucial, as timely intervention significantly impacts outcomes.

P65

EVALUATION OF CUTOFF CONCENTRATIONS IN DRUG OF ABUSE SCREENING ANALYSES

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Introduction: In hospitals, there is a need to screen for drugs of abuse (DOA) through qualitative and semi-quantitative determinations in urine. The methodology used is based on enzymatic (immune) assays. These assays allow for a fast preliminary result, and a confirmatory test may be required using quantitative chromatographic assays. The result of the screen tests is based on a cutoff concentration, where a positive result means that the sample has a concentration above the limit and a negative result means concentrations below the cutoff. The cutoff levels follow guidelines approved by official entities and are dependent on the analytical techniques used and the objectives. Therefore, in non-legal contexts, such as hospitals, higher cutoff values are suggested to avoid false positives, reflecting the strong impact of the defined values.

Aim: Analyze the results of DOA screening assays in a hospital clinical laboratory and evaluate the impact of defined cutoff concentrations.

Methods: One year evaluation of 1504 urine samples for 7 DOA assays (majority from emergency department) using the Alinity®c (ABBOTT) analyzer: amphetamines (AP), barbiturates (BAR), benzodiazepines (BEZ), cocaine (COC), cannabinoids (CAN), methadone (MET) and opiates (OPI). The cutoffs were 50 ng/mL for CAN, 200 ng/mL for BAR and BEZ, 300 ng/mL for MET, OPI and COC, 500 ng/mL for AP. The results were analyzed graphically using Microsoft Excel®.

Results: Among 1504 urine samples, 531 women and 973 men, aged between 1 and 99, was found that the majority of the tests (88%) showed negative results and 12% were “estimated positive” (EP). Considering the “EP”, three groups stand out with the highest percentage: BEZ (36%), CAN (24,7%) and COC (10%).

Conclusions: BEZ are a class of drugs widely consumed by the population, so these results might be expected. The same applies to CAN, where 75% of the samples were from young people aged 15-39. For COC, 45% of the positive samples were

from adults aged 40-65, showing the possibility of false positives due to cross-reactivity. AP follow the same logic, as 46% of the "EP" cases came from the 40-75 age group. For these three drugs, maybe it would be advantageous to carry out confirmatory tests to check for the possibility of false positives or to consider an increase in cutoff values, regarding the non-legal context.

P66

HEREDITARY SPHEROCYTOSIS - HOW TO ASSESS HYPERFERRITINEMIA IN A PATIENT WITH ALREADY TREATED IRON OVERLOAD?

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Introduction: Ferritin is a commonly requested laboratory test. Hyperferritinemia may indicate increased iron stores, but is more commonly associated with inflammation and as a result of ferritin released from hepatocyte damage as in liver disease. In disorders associated with ineffective erythropoiesis and chronic hemolysis, low hepcidin levels will increase iron absorption which can result in hyperferritinemia with iron overload.

Case report: 64 years-old man presented with a mild normocytic/normochromic anemia (hemoglobin=11g/dL) with marked hyperferritinemia (>1000ng/ml) and high transferrin saturation (TSAT=90%). Genetic test for hemochromatosis was negative and MRI showed mild to moderate hepatic iron overload and splenomegaly with signs of iron accumulation. Hemolytic anemia was investigated and the diagnosis of Hereditary Spherocytosis (HEs) was confirmed by a positive cryohemolysis test and a SDS-PAGE with a secondary protein deficit in spectrin and protein 4.2 resulting from a primary deficit in ankyrin. Next Generation Sequencing found no mutations in ANK1 gene, but compound heterozygosity for 2 mutations in SPTA1 gene justified the phenotype. Quantitative phlebotomies were initiated without patient's tolerance. Treatment switch to iron chelation resulted in an improvement of anemia (hemoglobin=12.4g/dL) with reduction of iron overload (TSAT=40% and ferritin=191ng/mL).

MRI was performed again and revealed normal iron concentration that allowed drug suspension. Three years later, an increase in ferritin (561ng/ml) was found in follow-up analysis. Without evidence of iron overload, after three months of alcohol abstinence and changed dietary habits, a ferritin correction was verified (356ng/ml), proving that hyperferritinemia was secondary to lifestyle habits.

Discussion: This patient had two contributors to hyperferritinemia, which should be managed differently. Recent guidelines do not recommend to re-treat patients once significant iron re-accumulation is not expected. On the contrary, high-calorie dietary habits and alcohol consumption are responsible for hyperferritinemia which is corrected by changes in lifestyle habits. Therefore, since hyperferritinemia is a non-specific finding, the cause of hyperferritinemia must be clarified in order to avoid unnecessary treatments. Individual cases can vary and a comprehensive assessment is crucial to determine the cause of hyperferritinemia in a patient treated for iron overload.

P67

WHEN ALL FACTORS BECOME UNDETECTABLE – A CASE REPORT OF DISSEMINATED INTRAVASCULAR COAGULATION

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Introduction: Disseminated intravascular coagulation (DIC) is a syndrome characterized by widespread intravascular deposition of fibrin, in such a scale that platelets and coagulation factors are consumed, resulting in bleeding; tissue ischemia from occlusive microthrombi may also occur.

Case Report: A 31-year-old woman, 36 weeks pregnant, smoker, with a past medical history of lower limb thrombophlebitis in previous pregnancy. She was admitted in the emergency department because of abdominal pain and leg pain. She was in labor and having deep vein thrombosis in lower limb. In the day before, fetal ultrasound was normal, including uterine artery and placental flows, as well as cardiotocogram. Her first analysis after admission indicated 12,3 g/dL of Hb (similar to her previous result two months before) and thrombocytopenia (87 000 platelets/μL; previous result 189 000), confirmed by observation of peripheral blood smear; PT (24,8 seconds; INR 1,98) and aPTT (41,6 seconds) were raised, D-dimers were extremely elevated (>200 000 ng/mL) and fibrinogen was undetectable (<50 mg/dL). By this time, clinical information stated fetal death in utero. One hour after the first analysis, Hb had lowered to 10,8, thrombocytopenia remained, and coagulation tests were completely abnormal (INR>12, aPTT >120s, D-Dimers >200 000, fibrinogen <50). During C-section, she was administered fibrinogen, fresh frozen plasma and tranexamic acid. Four hours after the second analysis, Hb lowered to 7,5 (with clinical information of DIC), platelets lowered (51 000), coagulation times were normal (INR 1,11; aPTT 23,1), D-dimers 498 760, fibrinogen 83, and by that time she was in Intensive Care Unit (ICU). During ICU stay, she was administered fibrinogen and erythrocyte concentrate, she had no further hemorrhagic or thrombotic events and, after resolution of acute renal lesion, she was clinically stable. During the following days, Hb and platelets improved, D-dimers lowered and coagulation times remained normal.

Discussion / Conclusion: Hemorrhage in DIC may be life-threatening, therefore it is a medical emergency. Coagulation tests may have extremely abnormal results, such as seen in this case. Quick recognition of the situation and reporting to clinical staff is essential.

P68

WHEN SYNOVIAL FLUID IS… BLOOD

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Introduction: Although the main cause of hemarthrosis is trauma, atraumatic hemarthrosis also occurs, often related to hematological pathology associated with higher risk of hemorrhage, or with other causes, some rare, namely anticoagulants, including direct oral anticoagulants (DOAC).

Case report: A 75-year-old woman with history of paroxysmal atrial fibrillation, medicated with edoxaban 60 mg id, and with knee osteoarthritis, presented with acute pain and swelling of knee, without history of recent trauma.

Through ultrasound-guided knee arthrocentesis, 50 mL of intra-articular hematic fluid was aspirated and sent for cytological and microbiological analysis. Analytical study (including complete blood count, coagulation tests, liver and kidney function tests, NT-proBNP and C-reactive protein) and x-rays of knee and surrounding bones were also performed.

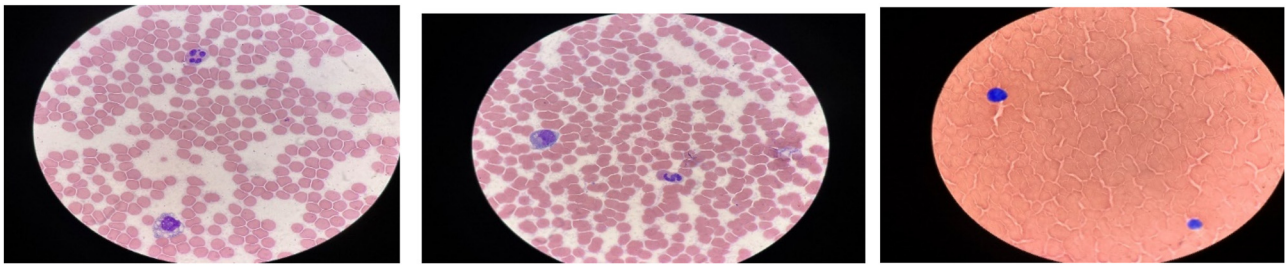
Analytical study only revealed raised aPTT (50,7”), as expected in patient under DOAC.

In cytological synovial fluid analysis: synovial fluid blood-red and less viscous than normal; cell count 690 cells/mm3. In microscopic examination: mostly polymorphonuclear cells, with differential count of 66% neutrophils, 18% lymphocytes and 16% monocytes; very abundant erythrocytes; no uric acid crystals.

In microbiological analysis: no microorganisms. Radiographs: knee osteoarthritis; absence of fractures or tumors.

Discussion: Synovial fluid can be normal, non-inflammatory, inflammatory, septic or hemorrhagic. Normal synovial fluid is macroscopically transparent, clear, straw yellow and viscous; nucleated cell count is 100-200/mm3; microscopical examination reveals mostly mononuclear cells (<25% polymorphonuclear cells).

In this case, synovial fluid was blood-red and less viscous; cell count was higher; microscopic examination showed mostly polymorphonuclear cells (reflecting peripheral blood differential count), and very abundant erythrocytes. In every aspect, macroscopically and microscopically, this synovial fluid was extremely similar to blood, so it was hemorrhagic. The cytospin technique usually used for microscopic examination wasn't adequate; thin blood smear technique was more useful. Thus, this reports an atraumatic hemarthrosis related to oral anticoagulation, a rare complication of DOAC.



P69

INTERFERENCE OF INTRAVENOUS DEXAMETHASONE ON THE SERUM CREATININE

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Case Report: This case report concerns a 52-year-old man with malignant neoplasia of the oropharynx undergoing palliative chemotherapy. A blood sample collected was processed on Beckman Coulter® AU5800 equipment using the kinetic colorimetric method yielded a serum creatinine value of 7767 umol/L [59-104], approximately 74 times the upper reference threshold. There was no increase in serum urea or changes in the ionogram, and there was no history of similar values or clinical conditions that would justify this result. The sample was then processed again on another equipment, the Atellica® CH analyzer using the enzymatic colorimetric method, and a similar value was obtained.

Due to the inconsistency of the results, a new sample was requested, revealing a serum creatinine close to the patient's baseline value. Prior to this case, three other patients had experienced similar circumstances.

Discussion: The significant increase in creatinine levels over a relatively short period, along with the normal urea levels, raised suspicions of a spurious result. A new sample was requested to confirm the results and repeating the creatinine analysis by different methods (colorimetric and enzymatic), both of which yielded similar results. Consultation with nursing revealed that the patient had only received intravenous dexamethasone as treatment. This specific brand that was administered contained creatinine as an excipient. The medication was administered as a bolus via a cannula in the dorsum of the hand, and the blood sample was drawn afterwards by venipuncture of the same arm.

In conclusion, we would like to raise awareness that spuriously high creatinine results may occur due to contamination of the sample with some brands of intravenous dexamethasone. This is not due to analytical interference but rather the correct measurement of creatinine. This serves as a general reminder that samples for analysis should not be drawn near the injection site immediately after intravenous drug administration and is a good example of the significant impact pre-analytical factors can have on result accuracy. Therefore, this finding made it possible to alert clinicians to similar situations of elevated serum creatinine levels due to an exogenous substance rather than actual renal dysfunction or methodological interference.

P70

AUTOIMMUNE HEPATITIS - A CASE REPORT

Patricia Achando[1], Ana Margarida Ribau[1], Elva Cardoso[1], Priscila Silva[1]

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Autoimmune hepatitis (AIH) is a chronic inflammatory disorder characterized by loss of tolerance towards hepatic auto-antigens, leading to an autoimmune attack to the liver.

AIH affects all ages and races, and is subdivided into type 1 (AIH-1) and type 2 AIH (AIH-2): AIH-1 is by far more common and affects both children and adults, whereas AIH-2 is mainly a pediatric disease.

The diagnosis of AIH is based on histological abnormalities, characteristic clinical and laboratory findings (elevated serum aspartate aminotransferase [AST], alanine aminotransferase [ALT], increased serum IgG concentration), and the presence of one or more characteristic autoantibodies.

Circulating autoantibodies have come to play a significant role in the diagnosis of AIH. (Table 4)

AIH-2 is characterized by positive anti-liver kidney microsomal antibody type 1 (LMK1) and/or anti-liver cytosol type 1 (LC-1) antibody.

Case: A previously healthy 19-year-old man, presented to emergency department with strong head pain and referring loss weight. He had an increased liver enzyme levels, **AST** 87U/L and **ALT** 175 U/L.

The patient had no medical or family history of hepatic disease. He was forwarded to an Internal Medicine appointment for further diagnosis. His laboratory findings were showed in analytical report.

The patient is currently waiting for liver biopsy but was already diagnosed with AIH Type 2.

Considerations: As the prevalence of AIH is increasing in all the patients with non-specific symptoms of the liver disease, AIH should be considered more often during diagnosis.

AIH Type 2 occurs mostly in children, presents worse clinical course, and requires long-term care. Liver biopsy is a valuable tool in diagnosis, prognosis and therapeutic management decisions in patients with liver disease. A complete biochemical response is defined as normalization of serum transaminase activity and IgG level, which should be achieved no later than 6 – 9 months after treatment initiation. It will be interesting to follow this case.

The early diagnostics of AIH is extremely relevant in the disease's monitoring and the laboratory can play an important role.

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IgA	**304**	mg/dL	70 – 400
IgG	**2140**	mg/dL	650 – 1620
IgM	**122**	mg/dL	30 – 265

Anticorpos anti-nucleares e citoplasmáticos – IFI

Núcleo:	**Negativo**
Nucleolos:	**Negativo**
Metafases:	**AC-26- Fuso mitótico (NuMA-1) (1/320)**
Citoplasma:	**Negativo**

Perfil Hepático (Immunoblot)	**Positivo. Integrar e valorizar resultado no contexto clínico e de outros meios complementares de diagnóstico.**
AMA-M2	**Negativo**
AMA BPO	**Negativo**
gp 210	**Negativo**
Sp 100	**Negativo**
PML	**Negativo**
LKM-1	**Negativo**
LC-1	**Positivo +++(forte). Padrão de imunofluorescência também sugestivo de positividade para Ac. anti-LC-1 (titulação: 1/320).**
SLA/LP	**Negativo**
Ro-52	**Negativo**

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SLA/LP	**Negativo**
Ro-52	**Negativo**

P71

MALA (METFORMIN-ASSOCIATED LACTIC ACIDOSIS) – ABOUT TWO CASES IN CENTRO HOSPITALAR BAIXO VOUGA

Patrícia Achando[1], Ana Margarida Paradela[1]

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Metformin is a biguanide compound prescribed as first-line therapy for the treatment of diabetes mellitus in patients with no renal disease.

Metformin-associated lactic acidosis (MALA) is a rare complication of altered lactate and hydrogen metabolism defined as pH < 7.35 and lactate > 5.0 mmol/L in the setting of metformin use or overdose, it carries a mortality rate of up to 50%.

A predisposing pathophysiological condition (acute or chronic medical comorbidities) leads to accumulation of metformin in the bloodstream and subsequent hyperlactatemia and metabolic acidosis.

It is difficult to find the etiology of an acute presentation of lactic acidosis but it is possible to measure the level of metformin. When the concentration is >5 µg/ml we are in the presence of MALA, however only a few laboratories do this measurement.

Case 1: Man 73 years old, autonomous and independent, presented to emergency room with disjointed speech. He had acute renal disease and diabetes mellitus treated with Dapaglifozin/Metformin 5/1000mg 2id. His laboratory findings were showed on figure 1.

He was admitted in intensive care unit to start mechanical ventilation and hemodialysis. During hospitalization he had sepsis by *Proteus mirabilis* and tracheobronchitis by *Enterococcus faecalis*, both solved, died after 65 days of hospitalization.

Case 2: Woman 84 years old, autonomous and independent, presented to emergency department with several dyspnea. She had diabetes mellitus treated with metformin/sitagliptin 1000/50 mg 2 id. Her laboratory findings were showed on figure 2.

She was admitted in intensive care unit to start hemodialysis, died after 5 days of hospitalization.

Considerations: The possibility of MALA should be considered in all of patients treated with metformin.

The prognosis was strongly correlated with the presence of kidney, heart, and liver failure, sepsis, and multi-drug intoxication.

Hemodialysis exhibits a higher clearance rate of metformin and lactate however the literature is not clear about the use of this technique on MALA patients. It's important to define the best way to treat MALA. Regardless of the rarity of severe metformin intoxication, it assumes a role of great importance due to the vast number of patients potentially at risk.

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Day 0

Gasimetria arterial com Co-oximetria			
pH	**6.915**		7.350 – 7.450
pCO2	**23.3**	mmHg	35.0 – 45.0
pO2	**98.1**	mmHg	80.0 – 100.0
Na+	**133**	mmol/L	135 – 148
K+	**5.9**	mmol/L	3.5 – 5.3
Cloro	**99.0**	mmol/L	98.0 – 106.0
Ca++	**1.11**	mmol/L	1.13 – 1.32
Glu	**486**	mg/dL	65 – 110
Lac	**12.0**	mmol/L	< 1.8
HCO3–	**4.6**	mmol/L	22.0 – 28.0
HCO3std	**5.0**	mmol/L	
TCO2	**5.3**	mmol/L	23.0 – 28.0
BE(B)	**-25.7**	mmol/L	-2.0 – 3.0
SO2c	**92.30**	%	
HTC	**18.0**	%	37.0 – 47.0
THbc	**6.0**	g/dL	14.0 – 18.0
Fracção O2 Hb	**91.1**	%	> 96.0
Fracção CO Hb	**0.9**	%	< 2.0
Fracção Met Hb	**0.4**	%	< 1.5
Fracção H Hb	**7.6**	%	0.0 – 5.0
Hiato aniónico	**35.20**	mmol/L	8.00 – 16.00

Ureia	**126.6**	mg/dL	19.0 – 51.0
Creatinina	**6.20**	mg/dL	0.70 – 1.30
TFGe (MDRD)	**9**	mL/min./1.73 m^2	> 60

Analytical report - Case 1

Day 0

Gasimetria arterial com Co-oximetria			
pH	**6.716**		7.350 – 7.450
pCO2	**35.0**	mmHg	35.0 – 45.0
pO2	**349.1**	mmHg	80.0 – 100.0
Na+	**136**	mmol/L	135 – 148
K+	**6.7**	mmol/L	3.5 – 5.3
Cloro	**96.0**	mmol/L	98.0 – 106.0
Ca++	**1.24**	mmol/L	1.13 – 1.32
Glu	**384**	mg/dL	65 – 110
Lac	**9.0**	mmol/L	< 1.8
HCO3–	**4.4**	mmol/L	22.0 – 28.0
HCO3std	**3.6**	mmol/L	
TCO2	**5.5**	mmol/L	23.0 – 28.0
BE(B)	**-30.8**	mmol/L	-2.0 – 3.0
SO2c	**99.50**	%	
HTC	**32.0**	%	37.0 – 47.0
THbc	**11.0**	g/dL	12.0 – 16.0
Fracção O2 Hb	**98.8**	%	> 96.0
Fracção CO Hb	**0.3**	%	< 2.0
Fracção Met Hb	**0.4**	%	< 1.5
Fracção H Hb	**0.5**	%	0.0 – 5.0
Hiato aniónico	**42.20**	mmol/L	8.00 – 16.00

Ureia	**182.1**	mg/dL	19.0 – 51.0
Creatinina	**7.83**	mg/dL	0.50 – 1.10
TFGe (MDRD)	**5**	mL/min./1.73 m^2	> 60

Analytical report - Case 2

P72

PRECISION IN THERAPEUTICS: REVEALING OFF-TARGET DOSING AND VARIABILITY IN NEW ANTIEPILEPTIC DRUGS

Paula Leal[1], Carlos Costa[2], Cláudia Fernandes[1], Anabela Carvalho[1], Alcina Mateus[1], Júlia Matos[3], Cláudia Barata[3], Teresa Reis[1], Marília Rocha[3], Eulália Costa[1], Fernando Rodrigues[1]

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[2]*Unidade Local de Saúde da Lezíria, Serviço de Patologia Clínica,*
[3]*Unidade Local de Saúde de Coimbra, Serviço de Farmácia Hospitalar / HUC*

Introduction: Antiepileptic drugs (AEDs) are vital for epilepsy managing, yet their heterogeneity and complex pharmacokinetics (PK) present challenges. There is significant inter-individual variability in response to AEDs, and while it would be beneficial to exclude a pharmacogenomic cause, consideration of the drug's PK profile is essential. Our laboratory expanded testing to 8 new AEDs via LC-MS/MS in April 2023, supplementing existing methodologies.

Objective: We aim to showcase the importance of monitoring and interpreting dosages of new AEDs (lacosamide, lamotrigine, levetiracetam, perampanel, zonizamide, topiramate, eslicarbazepine and oxcarbazepine) in collaboration with clinical and pharmaceutical services.

Materials and Methods: Retrospective study. The AEDs were quantified by LC-MS/MS (Waters Acquity™) from 24/04/23, 31/01/24. Descriptive statistics were performed using *Microsoft Excel*®. Pre-analytical conditions were assumed to be met. Therapeutic ranges and classification of results as sub-therapeutic, therapeutic, or supra-therapeutic were established according to international consensus papers (1)(2).

Results: The study analysed 607 requests from 356 patients, 45.5% male and 54.5% female (3-101 years old). Most requests (67%) came from the outpatient clinic of the Epilepsy and Sleep Monitoring Unit, Refractory Epilepsy, and the Emergency Department. Off-target dosages were found for all AEDs. In total, 19.2% were sub-therapeutic and 12.1% were supra-therapeutic.

Conclusions: There is considerable variability of results within each AED. Approximately 30% of all measurements were off-target. We emphasize the importance of quantifying AEDs and interpreting them regarding each patient response. Therapeutic drug monitoring improves patient safety, while providing better patient care with cost-benefit implications.

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P73

IMPORTANCE OF THE SAMPLE MATRIX IN THE MEASUREMENT OF TOTAL BILE ACIDS: COMPARISON OF TWO ENZYMATIC ASSAYS

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Introduction: Total Bile Acids (TBA) measurement works out as a crucial biomarker of liver function and hepatobiliary health, particularly in conditions as the intrahepatic cholestasis in pregnancy. TBA comprises a collective of over 20 bile acids synthesized by the liver from cholesterol. The enzymatic determination of TBA, by 3α-hydroxysteroid dehydrogenase,

remains the predominant analytical method due to its simplicity and accessibility, as well as its adaptability to automated analyzers. In this study the authors evaluate the performance of two enzymatic assays (EA) for the measurement of TBA, also considering the HIL serum quality index.

Objective: To compare TBA results and the clinical performance of two EA, using the BioSystems® (BS) and the Abbott® (AbT) assays.

Materials and methods: Eighty-six (86) fasting serum samples were analyzed, with both assays to measure TBA concentrations. Statistical analysis involved paired sample T-test and Pearson's correlation test, using the SPSS Statistics (IBM SPSS). For the clinical performance, the reference range established was ≤ 6.0 µmol/L, based on fasting values. Sample HIL index serum was determined in the Abbott® Alinity c analyzer.

Results: Similar means and range results were found: 20.8 µmol/L for BS (2.3-119.8 µmol/L) and 19,0 µmol/L (1.0-163.0 µmol/L) for AbT. The correlation study revealed a positive, and moderate correlation (r=0.592) and no statistically significant differences were observed between paired results (t=0.723; p>0.05). Regarding clinical decision, BS exhibited 79.1% results > 6.0 µmol/L compared to AbT's 52.3%. In 23 samples (26.7%) clinical interpretation revealed disagreement between assays, with higher results in the BS assay. In these discrepant samples, serum index revealed 9 samples (39.1%) with lipemia and 2 with hemolysis.

Conclusions: The observed differences between the assays were not statistically significant, showing that both are suitable for TBA quantification. About 1/3 of the pathological samples were discordant in clinical interpretation which can be attributed to intrinsic sample characteristics, like variable lipid content of the matrix, revealing the importance of pre-analytical conditions. In the presence of abnormal TBA results both clinical and laboratory findings should be considered.

P74

CRYOGLOBULINEMIA UNVEILED: THE ROLE OF PERIPHERAL BLOOD SMEAR IN HAEMATOLOGICAL ANOMALIES ANALYSIS

Paulo Silva[1], Nuno Silva[1], Fernando Rodrigues[1]

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Case report: A 91-year-old female was admitted to the neurology department due to a Total Anterior Circulation Stroke Syndrome. During hospitalization, she developed systemic complications from a bacterial pneumonia. As part of the routine clinical monitoring, a complete blood count was performed showing leukocytosis (11.44×10^9 cells/L) and normal values of hemoglobin and platelets count. Anomalies in the white cell nucleated (WNR) and the leukocytes differential (WDF) scattergrams from the automated analyzer Sysmex XN-9000 prompted further investigation through a Peripheral Blood Smear (PBS). The smear exhibited rare cells with morphology suggestive of plasmablast/proplasmocyte, a significant presence of disrupted cells, and a substantial amount of amorphous, blue-violet material, suggestive of cryoglobulin precipitates. To qualitatively confirm the cryoglobulin presence, the blood sample collected to the EDTA tube was observed immediately after heating (37ºC for an hour) and upon cooling to room temperature. It was verified the absence of deposits in the heated sample and their appearance after cooling, supporting the cryoglobulinemia hypothesis.

Discussion/conclusion: The presence of serum cryoglobulins is commonly associated with various medical conditions, including infectious diseases, and may cause inaccurate leukocytes or platelet counts, requiring a careful evaluation of these parameters.

Since cryoglobulins are immunoglobulins that typically precipitate at temperatures below 37ºC, heat processing of the sample is recommended to provide reliable results. This case underscores the importance of a PBS as a complementary diagnostic exam when scattergram abnormalities are observed. It often initiates further exploration into possible underlying health issues and helps ensure the delivery of more accurate results to clinicians.

P75

CASE REPORT OF INFECTIVE ENDOCARDITIS CAUSED BY GRANULICATELLA ELEGANS

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Infective endocarditis is a relatively uncommon but life-threatening disease of the endocardium of the heart, the three most common causes worldwide being staphylococci, streptococci and enterococci. Endocarditis due to *Granulicatella elegans* is a rare but clinically significant condition, as it represents a diagnostic challenge and often presents with frequent complications despite the use of appropriate antibiotic regimens. The current case describes a 79-year-old female patient with a prosthetic aortic valve implanted 9 months earlier. The patient presented with headache, dysarthria, emesis and atrial fibrillation with rapid ventricular rate. On admission, she was apyretic and denied any recent episodes of pyrexia. Laboratory tests revealed high troponin and NT-proBNP levels and the transthoracic echocardiogram identified a vegetation on the mitral valve. The blood cultures turned positive 20 hours after being collected. An isolate was identified as *G. elegans*, after which the antibiotic treatment was switched according to the 2015 ESC Guidelines for the management of infective endocarditis with a favorable outcome for the patient.

P76

GANGLIOSIDOSIS GM1 - REGARDING TWO CLINICAL CASES

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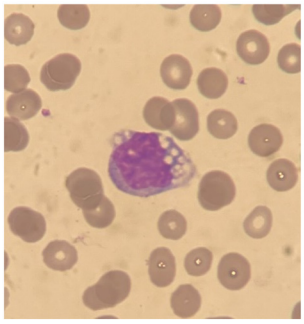
Introduction: GM1 gangliosidosis (GM1) is a rare lysosomal storage disease (LSD), defined by the deficiency of beta-galactosidase (β-Gal) enzyme activity. We present two case reports that highlight the clinical features and diagnostic challenges associated with this disorder.

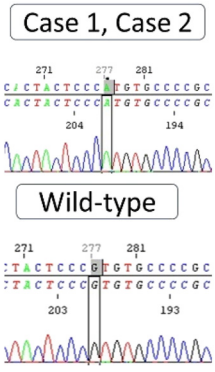
Case presentation: Case 1: 8-month-old female infant, born to first cousins. Personal history (PH) includes facial dysmorphism, developmental delay. Analytical study revealed anemia and 27% of lymphocytes with vacuolated cytoplasm. Presence of a cherry-red spot by fundoscopy. Dry blood sample enzyme assay revealed β-Gal activity deficiency - 1.95 pmol/h/puncture (4.51-32.27), that was confirmed in whole blood leukocyte extracts (BLE) - β-Gal 4 nmol/h/mg protein (73-585), suggestive of GM1. *GLB1* gene sequencing revealed the presence in homozygosity of the genetic variant c.176G>A (p.Arg59His), described as causative of GM1. The patient died at 11 months-old (2023).

Case 2: 9-month-old female infant, born to third cousins. The PH includes low-set ears, mongolian spots and bilateral deafness. Analytical study revealed anemia and lymphocytes with vacuolated cytoplasm. Presence of a cherry-red spot by fundoscopy. The results of qualitative tests in analysis of urinary oligosaccharide and sialyl-oligosaccharide by chromatography resulted in the GM1 pattern. The activity deficiency of β-Gal 7 mmol/h/mg protein in BLE is suggestive of GM1. *GLB1* gene sequencing revealed the presence in homozygosity of the genetic variant c.176G>A (p.Arg59His), confirming GM1. The patient died at 15 months-old (2016).

Discussion: Through familial background investigation, case 1 was found to be a niece of case 2, whom had been studied 8 years earlier and both were of gypsy ethnicity. Case 1 family did not receive prenatal counseling nor genetic testing as recommended probably due to lack of awareness or misinformation during pregnancy. These two family related patients illustrate the complexity of the clinical manifestations of GM1. Both cases presenting with lymphocytes with vacuolated cytoplasm and cherry-red macula on FE are red flags for an LSD. These cases highlight the challenges of an early diagnosis and the importance of hematologic diagnostic tests as first line investigations. The lack of effective treatments and the

severity GM1 emphasize the importance of genetic counseling. The genetic carrier studies and prenatal screening are highly recommended to prevent recurrence of GM1 in this family.





P77

PRENATAL SCREENING: WORKFLOW OPTIMIZATION THROUGH AN IN-HOUSE APPLICATION

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Introduction: Prenatal Screening (PS) is a crucial area within the Hormonology sector of our hospital. It involves calculating 1st and 2nd trimesters prenatal screenings from three maternity units, corresponding to 5 000 samples per year. In order to optimize the workflow of this area, an in-house application was developed.

Objective: To describe the PS workflow optimization through an in-house application.

Workflow design: from fallible to optimized: Regardless of the form, the main principles of the PS workflow are somewhat constant. It begins with the Obstetrician request in Clinidata XXI that is received by the Clinical Pathology Department (CPD). Then, the personnel responsible for the PS organize the received requests according to the scheduled ultrasound date, planning each workday according to the predefined organization. After the execution of the calculations in the dedicated PS software, the results are inserted in Clinidata XXI.

Before the development of the aforementioned application, most of the workflow regarding PS relied on paper support and manual transcription of data between the various software platforms used.

The development of this in-house application eased the process and allowed the automatization of some crucial steps: worklists by ultrasound date started to be generated in our LIS, avoiding human intervention; the conclusion of each report became registered informatically, excluding the need of its registration on paper support, with all its associated questions; transmission of the generated data in the dedicated PS software directly to Clinidata XXI, reducing the number of actions required to achieve the same end result.

Discussion: The development of this application undoubtedly improved the workflow of the PS area for various reasons, standing out the decrease for paper support, a reduction in the probability of occurrence of typing and transcription errors and a better control of the whole process.

Security concerns regarding the use of data by an external application could be raised, however, since no data is saved by the application, that does not come into question.

Conclusions: This in-house application, designed according to the needs of our CPD, allowed PS personnel to focus more on critical thinking tasks by saving their time and effort from monotonous actions.

P78

TEMPORAL TRENDS IN GLYCAEMIC CONTROL AMONG DIABETES MELLITUS PATIENTS (2012-2022) AND THE IMPACT OF THE COVID-19 PANDEMIC, PORTUGAL

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Introduction: Diabetes mellitus (DM) is a chronic disease characterized by sustained hyperglycaemia. The International Diabetes Federation reported an 8.5% prevalence of DM in 2013, which increased to 10.5% by 2021 in Europe. Also, it predicted that the global DM rate in 2045 will be 12.5%. In March 2020, the World Health Organization (WHO) declared the COVID-19 infection a pandemic. Recent research has shown that acute infection with SARS-CoV-2 can exacerbate hyperglycaemia, and uncontrolled DM can lead to more severe COVID-19 infections.

Objectives: To analyse the glycaemic evolution in DM patients, from the Northeast Lab database in a decade (2012 to 2022), and the impact of two years of pandemic.

Methods: All Glycated Haemoglobin (HbA1c) values from the beginning of the decade (2012/13), before the pandemic (2017/18), and after the declaration of the pandemic (2021/22) were retrospectively analysed. Diabetes was defined as a HbA1c value ≥6.5%, by WHO guidance. Data normality was assessed with the Kolmogorov-Smirnov test. Mann-Whitney and Kruskal-Wallis (post-hoc Bonferroni correction) tests were used to assess differences between groups.

Results: 10520 HBA1c results were analysed between 2012/13, 2017/18 and 2021/22, Of these, 39.2%, 30.5% and 31.4% had DM, respectively. The median (P25-P75) HbA1c decreased over the decade: 7.7 (7.0-8.8) % in 2012/13, 7.4 (6.8-8.3) % in 2017/18, and 7.3 (6.8-8.2) % in 2021/22. There were statistically significant differences between 2012/13 and 2017/18 ($p < 0.001$) and between 2012/13 and 2021/22 ($p < 0.001$). In 201/13, HbA1c levels (median [P25-P75]) in 45-64 years old individuals (8.0 [7.1-9.1] %) were higher than 2017/18 (7.4 [6.9-8.4] %; $p < 0.001$) and 2022/23 (7.5 [6.8-8.2] %; $p = 0.003$). Similar trend was observed for individuals ≥65 years old. There were no statistically significant differences between female and male individuals.

Conclusion: While the history of COVID-19 in this population is unknown, a slight increase (from 30.5% to 31.4%) in DM and the evolution of HbA1c levels during the pandemic do not suggest an increase in prevalence or a worsening of glycaemic control due to COVID-19. The significant decrease in HbA1c over the entire decade suggests a better control of DM, presumably through improved healthcare and lifestyle changes in these patients.

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ASSESSING GLOMERULAR FILTRATION RATE: A COMPARATIVE ANALYSIS OF TWO ESTIMATION FORMULAS

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Introduction: Glomerular filtration rate (GFR) is an indicator of kidney function, reflecting the rate at which plasma is filtered through the glomerulus into Bowman's space over a defined period and is often considered the best overall index of kidney function in health and disease. Estimating GFR in routine clinical practice relies heavily on equations, with serum creatinine being the primary biomarker utilized. However, selecting the appropriate equation for GFR estimation poses a challenge for clinical laboratories due to the availability of several options. Two equations commonly used are the Modification of Diet in Renal Disease (MDRD) equation with Isotope Dilution Mass Spectrometry (IDMS) and the Chronic Kidney Disease Epidemiology Consortium (CKD-EPI) 2009 equation, the latter updated in 2021. This equation is very similar to the

previous CKD-EPI equation published in 2009 but modified without applying a race coefficient factor. The CKD-EPI equations are the ones with the highest consensus and, according to the literature, greater accuracy, especially when cystatin C is also included.

Objective: To compare two equations for estimating GFR, the MDRD-IDMS and CKD-EPI 2021. The first is adapted to standardized creatinine methods and considers serum creatinine concentration, age, sex, and race, while the second, with the same variables, is independent of race.

Materials and Methods: The creatinine results of 7988 patients of both genders, aged 18 and older, were used, excluding the pregnant population. GFR was calculated using both equations.

Results: In 6274 patients, GFR was greater than 60 mL/min/1.73m^2 in both equations. In the range of results below 60 mL/min/1.73m^2, it was found that 3.7% (296) of patients had results with MDRD-IDMS between 51.2 and 59.9 mL/min/1.73m^2 and with CKD-EPI 2021 between 60.1 and 70.3 mL/min/1.73m^2. These results suggest an underestimation of GFR when using the MDRD-IDMS equation.

Conclusion: The results found highlight the limitations of both equations, as the underestimation of GFR with the MDRD equation is already known. In the case of using CKD-EPI 2021, there are also some limitations already published regarding the application of this equation in the European population. Determination of cystatin C and the use of the formula including cystatin C may help in the accuracy of the diagnosis. All GFR equations remain in estimation of GFR, especially rough at the individual level.

P80

SYPHILIS BEFORE FIFTIES – CURRENT TRENDS

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Introduction: Syphilis is a sexually transmitted infection caused by the bacterium *Treponema pallidum*. The infection can be acquired during sexual activity by direct contact and also from mother-to-baby transmission during pregnancy.

According to the World Health Organization and European Centre for Disease Prevention and Control (ECDC), the 2020 prevalence of syphilis cases exceeded 22 million people worldwide.

Aim: This study aimed to characterize new syphilis cases from patients of Centro Hospitalar e Universitário de Coimbra (CHUC), aged between 15-49 years, over a decade.

Methods: A restrospective anonymised study was performed between the January 1st 2013 to December 31th 2023. Data from Treponemal Tests (TT) and Non-Treponemal Tests (TNT), performed according to the reverse algorithm for syphilis diagnosis, were collected from ClinidataXXI software.

For TT and TNT, CMIA - Abbott Alinity Syphilis TP and TNT Rapid Plasm Reagin (RPR) – BioSystems were used (respectively). According to manufacturer recommendations, samples were considered reactive for TT with antibody titters above 1 S/CO and for RPR with a titre ≥1:4.

Male and female patients with new positive TT and TNT were considered for the diagnosis of new syphilis cases.

Results: In this study, 87590 TT (n=53998 patients) and 4460 TNT tests (n=1351 patients) were performed. Results showed 1001 new syphilis cases, distributed between 759 males (75.8%) and 242 females (24.2%). Age-specific incidence was higher between 25-30 years amongst male patients and between 30-35 amongst female patients.

A peak of new cases was observed in 2014, while 2016 was the year with least number of new cases. Overall, an increase of 131% was verified in the number of new cases over the past 7 years.

Discussion and Conclusion: Considering the collected data, female patients were more tested, probably because syphilis testing is part of the routine prenatal screening. However, the incidence amongst male patients was much higher. Considering the number of cases in patients under 50 years old, the most affected age group was between 25 - 30 years old.

In the last years the number of Syphilis screening tests performed in CHUC increased, particularly from the moment that the hospital HIV pre-exposure prophylaxis (PrEP) started in 2019, and this may contribute for the higher number of new cases.

P81

ABOUT A CLINICAL CASE: WHICH MARKERS SHOULD BE USED FOR THE DIFFERENTIAL DIAGNOSIS AND MONITORING OF HAEMOPHAGOCYTIC SYNDROME

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Introduction: Haemophagocytic syndrome (HS) is characterised by an hyperinflammatory state induced by macrophages and cytotoxic T cell which leads to a cytokine storm. HS is rare in adults, and it has different causes, symptoms and prognosis, so it is essential an early and a differential diagnosis.

Objective: Identify serological markers for the differential diagnosis and monitoring of HS.

Material and methods: XN3100™, Sysmex™: haemoglobin (sodium lauryl sulphate), leukocytes, neutrophils, erythrocytes, platelets (fluorescence flow cytometry);

Hemosil™, ACL TOP 500™: d-dimer (latex immunoturbidimetry) and fibrinogen (coagulometric);

Abbott™, Alinity c™: triglycerides (glycerol phosphate oxidase), aspartate aminotransferase and alanine aminotransferase [NADH (without p-5'-p)], lactate dehydrogenase (LDH) [lactate to pyruvate (NADH)], c-reactive protein (CRP) (latex immunoturbidimetry);

Abbott™, Alinity i™: procalcitonin (PCT) and ferritin (FERT) (heterogeneous chemiluminescence assay).

Results and discussion: Male patient, 26 years old, diagnosed with HS, with severe respiratory symptoms, hospitalized in the intensive care unit at Hospital Sousa Martins, ULS Guarda, with agammaglobulinemia and a missense mutation in perforin 1 gene. The characterization was made according to Hscore and the Histiocyte Society's criteria. The patient presented at day 0 pancytopenia, multiorganic failure, hypofibrinogenemia, d-dimer elevation, hypertriglyceridemia, CRP 6.2 mg/dl (highest value 7.96 mg/dl), PCT 3.1 ng/ml (highest value) and FERT 84000 ng/ml (highest value 329000 ng/ml) as described in the literature. All the variations showed by different analytes are due to the therapeutic approach except FERT and LDH. These raised due to, respectively, hyperinflammation and the worsening of the pulmonary function. FERT increases can be explained by the cytokine storm and the impaired activity of the macrophages. The increment of LDH could be related with lung damage and probably due to lactate dehydrogenase isoenzyme 3. These concentration increase do not parallels the hepatic transaminases values.

Conclusion: Both FERT and LDH equally performed to differential diagnosis and monitoring HS associated with Hscore and Histiocyte Society's criteria.

P82

KINGELA KINGAE - A CASE REPORT

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Introduction: Osteoarticular infections (OAI) represent a significant source of morbidity, with particular importance in pediatric age due to their frequency and severity. *Kingella kingae* is a gram-negative, facultative anaerobic and beta-hemolytic coccobacillus, which has emerged as the predominant causative agent in osteoarticular infections in the pediatric population, particularly among children under 4 years old.

Case report: A 12-month-old girl presented to the emergency department because her mother noticed that she was not using her right arm to play or pick up objects. The patient had experienced acute median otitis two weeks before this episode. No significant past medical history was refered. The toddler is fully vaccinated and attends to day care facility.

In the emergency department, the patient exhibited a C-reactive protein level of 2.5 mg/dL and an erythrocyte sedimentation rate of 53 mm/hr. During the physical examination there was noted lentification in wrist mobilization with a limitation of pronation. The wrist ultrasound revealed a mild increase in the thickness and echogenicity of the subcutaneous cellular tissue, indicative of edema, without the presence of fluid.

A blood culture bottle was collected and incubated in an automated blood culture instrument. After 28.3 hours of incubation, the blood culture yielded a positive result, and the gram stain revealed the presence of short Gram-negative rods. Subsequently, the blood sample was inoculated onto chocolate agar and incubated at 35°C with 5% CO2. After 36 hours of incubation, small colonies were observed on the chocolate agar. The isolate was identified as *Kingella kingae* using MALDI-TOF.

Antimicrobial susceptibility tests, following the EUCAST 2023 clinical breakpoints, indicated that the isolate was susceptible to ceftriaxone and cefuroxime but resistant to benzylpenicillin.

Discussion: It is now widely acknowledged that *K. kingae* is a common cause of bacteremia without a specific focus, being part of the HACEK group of organisms. In our laboratory, *Kingella kingae* has been isolated only twice in the last 20 years. The first instance occurred in the synovial fluid of a 7-year-old boy, and the second case is reported in this particular case.

The future of *Kingella kingae* diagnosis may involve molecular diagnostic techniques, potentially enhancing the sensitivity, specificity, and speed of detection in cases where traditional culture methods may be challenging or time-consuming.

P83

QUALITY INDICATORS AND THEIR IMPACT ON LABORATORY RESULTS IN A SIMULATED CLINICAL CASE

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Introduction: Quality indicators (QIs) obtained from the results of Internal Quality Control (IQC) and External Quality Assessment (EQA) in clinical laboratories are fundamental tools for quantifying the quality of results.

QIs make it possible to reduce the risk of errors affecting laboratory results and their impact on clinical decision-making.

Materials and methods: The clinical case simulated a 73-year-old patient who had been complaining of weakness and abdominal pain for 4 months. She was taking naproxen (anti-inflammatory). The laboratory results requested indicated: hypochromic microcytic anaemia with iron deficiency, (Hb = 9.8 g/dL, VGM = 72fL, and Iron = 15µg/dL), November 2023.

Management indicators were analysed: maintenance of equipment, calibration, batches and expiry dates of reagents and control material, and QI results for Hb and Iron: Coefficient of Variation (CV%), Bias%, Deviation Index (DI), Total Analytical Error (TE%) and Measurement Uncertainty (MU%). Analytical Performance Specifications (APS) based on biological variability (EFLM) were used.

Results and Discussion: In 2023, for Hb and Iron, the batches of reagents and control material were within the expiry date, and the maintenance and calibration plans were complied with.

Hb – IQC: 11 batches of 3 concentration levels (4.37 g/dL, 11.35 g/dL, 15.45 g/dL).

EQA: 32 samples, 10 of which ranged from 6 to 10.6 g/dL in concentration, with good results.

Iron - IQC, 2 batches of 2 concentration levels (112.3µg/dL, 248.3µg/dL); EQA, 17 samples, 7 of which ranged from 11.99 to 25.96 µg/dL in concentration, with good results. A low concentration level in IQC was not used to assess the patient's result (15µg/dL).

The optimal APS were met: Hb (CV% = 0.66; Bias% = - 0.17, TE% = 1.26 and MU = 1%); average annual DI = -0.05 (Excellent);Iron (CV%=3.65; Bias% =3.2, TE%= 9.2 and MU =7%); average annual DI = 0.29 (Excellent).

Conclusion: The QIs assessed (CV%, TE%, Bias%, MU%) for the parameters Hb and Iron complied with the optimum APS of the EFLM, and it can be inferred that the analytical process is controlled in terms of precision and accuracy. However, in the case of iron, a low concentration level could be applied in the IQC to cover the entire range of analyses. The laboratory results issued allowed for correct clinical diagnosis and adequate therapeutic monitoring.

P84

MACROTHROMBOCYTOPENIA, A RARE CAUSE OF THROMBOCYTOPENIA

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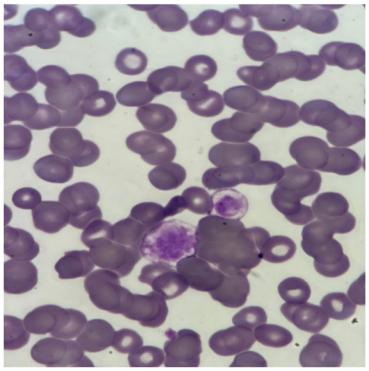
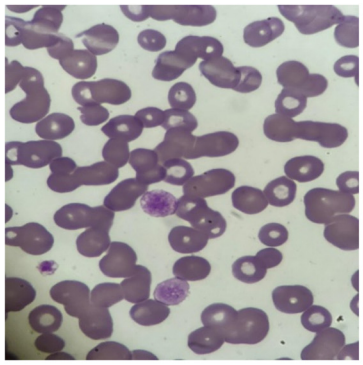
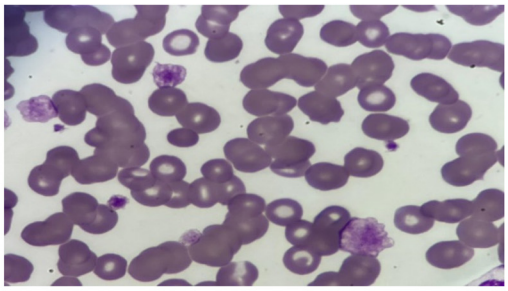
Introduction: Thrombocytopenia is a relative common finding on total blood count, but inside this one we have a rare condition, estimated less 0,5%, called macrothrombocytopenia. This could happen due a hereditary medical condition, somatic mutations, proliferative and dysplasic syndromes and even drugs like some chemotherapy.

Clinical Case: Man, 75 years old, former smoker (40 packs-year), moderate alcohol consumption, atrial fibrillation (hypocoagulated with warfarin), and history of subtotal gastrectomy in 2019 for gastric adenocarcinoma (pT1bG1N0R0). No adjuvant treatment needed. In July 2023, after an intramuscular injection in the right thigh, developed a significative hematoma requiring recourse to the ER. Analytically, with severe anemia (Hb 6,0g/dL) for which he underwent a transfusion of erythrocytes with a good response and a vitamin K antagonist. A month later, in a follow-up consultation, he maintained N/N anemia (Hb 10g/dL), 3% of Reticulocytes, normal RPI (2,7%) and without thrombocytopenia (PLTs 249.000/uL). He was sent to a hematology consultation due to persistence of this isolated anemia. He had a consultation in October, where he denied new blood losses, fever or B symptoms. New analytical study showed a progressive increase in Hb level (11,2g/dL) but now with thrombocytopenia (100.000/uL). The pathologist analyzed the blood smear which highlighted marked platelet anisocytosis, with numerous giant platelets, which prevents correct platelet measurement by impedance method. In this situation, the ideal would be to confirm the platelet count using optical fluorescence. This case is an example of pseudo-thrombocytopenia because the real value will certainly be higher. The patient was advised to change dietary habits and to suppress alcohol intake until further reassessment. In January 2024, Hb continued to increase (12,5g/dL), with slightly better thrombocytopenia (PLT 136.000/uL), but the smear maintained the presence of macrothrombocytes and with new finding dysplastic monocytes. Clinical pathologist once again highlighted these findings to promote the etiological study of this macrothrombocytopenia, which has so far no defined cause.

Conclusion: Macrothrombocytopenia is a very rare condition that needs further investigation especially when is moderate to severe. Pathology Laboratory play an important role on screening blood count and smear providing more information to the clinicians right from admission to all phases of investigation.

P85

"PRECISION ALLERGOLOGY" APPLYING A "TOP-DOWN" DIAGNOSTIC APPROACH IN A PATIENT WITH TREE NUT ALLERGY

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Tree nuts are one of the most common foods causing acute allergic reactions. The diagnostic algorithms traditionally start with the clinic history, followed by skin tests, then IgE assays adding allergen molecules for "component-resolved diagnostics".

History: Girl, 11 years: since she was 9 years old with complains of swelling of the lips, tingling and oral/pharyngeal itching after eating a nut chocolate, worsening over the years. In a given moment, her father was eating walnuts and she developed local hives two minutes after he touched her face.

Testing: Total IgE:135 UI/mL
ImmunoCAP® Specific IgE (kU/L): Peanut=0.01; Walnut=22,00; Hazelnut=5.01; Almond=2.45; Cashew=0.14; Pistachio=0.49
ImmunoCAP® ISAC: positivity only to Jug r 1=4 ISU-E
ALEX2®: detected sensitization to storage proteins from hazelnut, walnut, and macadamia. Total extract from macadamia and pecan were also positive. Total extract from almond revealed itself to be negative and none of the molecular allergens from pistachio was positive.

Oral food challenge was performed for both cashew and almond and resulted negative.

A tailored avoidance diet was introduced, which improved the quality of life.

Discussion: Detection of specific IgE in serum is strictly a marker of allergic sensitization.

Using molecular component tests some information are improved, such as: "analytical sensitivity" (particularly when important allergens are underrepresented or lacking in the extract) and "analytical specificity" (which provides additional information on potential risks, possible cross-reactivity or primary sensitization).

Molecular diagnosis using multiplex assays enables testing a high number of individual allergen molecules simultaneously, which represents an important screening tool to assess sensitization by identifying potential triggers of anaphylaxis.

In patients with nut allergy sensitized to storage proteins, the highest clinical reactivity is related to the botanical family, with strong correlations between cashew-pistachio and walnut-pecan-hazelnut-macadamia. Consequently, for the differential diagnosis of our patient, molecule-based sensitization tests revealed to be important in identifying the degree and potential clinical relevance of cross reactivity.

So, we can conclude that studying the allergic molecular profile of the patient is of extreme importance, in terms of a more accurate diagnostic, prognostic and therapeutic information.

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EVALUATION OF A FULLY AUTOMATED PLATFORM FOR CMV AND EBV VIRAL LOAD DETERMINATION IN PLASMA

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Cytomegalovirus (CMV) and Epstein-Barr virus (EBC) belong to the family Herpesviridae (HHvs), with an estimated global seroprevalence around 83% and 90% respectively. As with all HHvs, following primary infection, CMV and EBV characteristically establish a lifelong latent infection in the host, with the ability to reactivate.

The recognition of the clinical importance of CMV disease in the setting of immunodeficiency and immunosuppression, as well as the causative association of EBV in posttransplant lymphoproliferative disorders, has led to the implementation of clinical guidelines for screening, diagnosis, prevention, and treatment of CMV and EBV infections.

Quantitative real-time PCR (qPCR) is the gold standard for viral load (VL) determination being crucial for the identification of patients who are at risk for the development of CMV- and EBV-associated complications in the posttransplant setting, as DNAemia indicates viral replication.

The aim of this study was the evaluation of the Alinity CMV and EBV assays for VL determination in plasma.

A total of 72 clinical (47 CMV + 25 EBV) and 3 pairs of external quality control samples (ECQs) was tested with Alinity m platform and TibMolBiol qPCR (commonly used in routine diagnosis of CMV/EBV in our laboratory).
The correlation coefficient between VL obtained by the two methods was R^2=0.99 for CMV and EBV. The mean bias between assays (TibMolBiol minus Alinity) was -0.60 and -0.31 LogIU/mL for CMV and EBV quantification, respectively.

The concordance between assays was 70.2% (33/47 CMV samples) and 76.0% (19/25 EBV samples). Of the 12 discordant CMV samples that were negative with TibMolBiol, 9 were weakly positive with Alinity (35-595 IU/mL) and 5 were detected under the lower limit of quantification (<LLOQ). For EBV, from the 6 negative samples with TibMolBiol, 1 was weakly positive (24 IU/mL) with Alinity and 5 were detected <LLOQ. Considering the VL clinical limits, the differences found are not clinical significative. Additional, EQCs showed 100% concordance with the score reported.

In conclusion, the Alinity assay demonstrated high performance for VL determination of both viruses.

This automated platform allows the continuous processing with a reduction in operator errors allowing standardization of the process and to perform any test in any time. More, with a turnaround time testing of 2-3 hours the interval between diagnosis and clinical management of the patient can be dramatically decreased.

P87

PREVALENCE OF INTESTINAL PARASITES: A LONGITUDINAL STUDY FROM 2022 TO 2023 IN THE BGS GROUP

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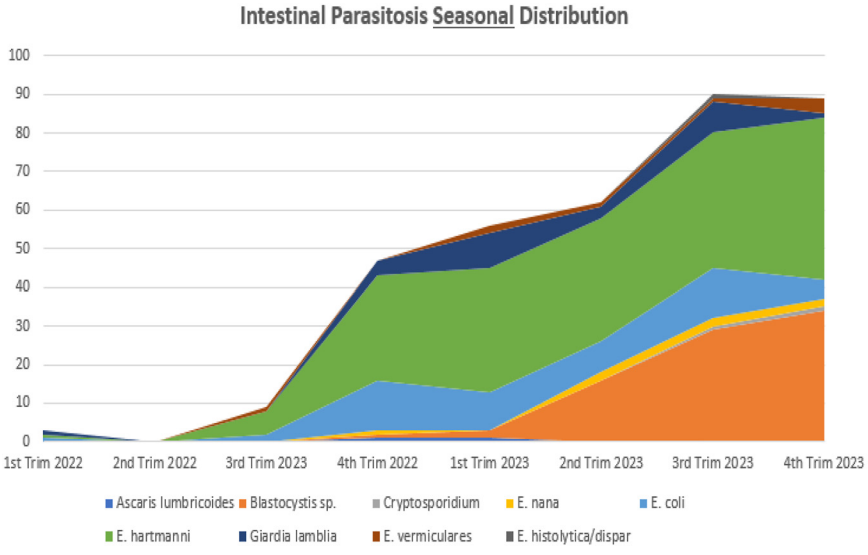
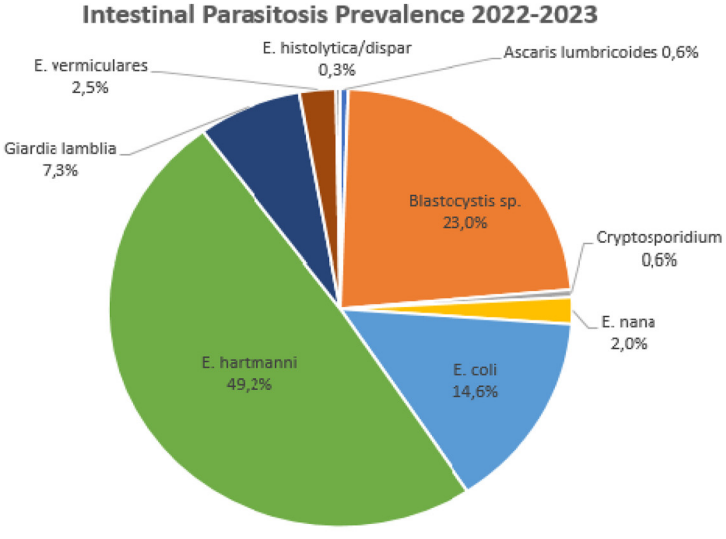
Introduction: The intestinal parasitoses (IP) are still being the cause of some gastrointestinal discomfort, diarrhea and weight loss at the population. In Europe these studies are rare and difficult to find.

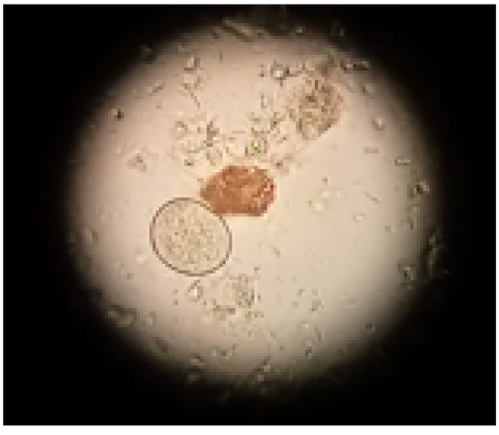
Objectives: Identify the parasites, study the gender and age that they affect and search their seasonality.

Materials/Methods: The study was performed from 2022-2023 in a group of 2615 individuals from 0-90 years, in a total of 6932 faecal samples at BGS. All the tests were required by a physician. Sample preparation was carried out using a mini Parasep SF Faecal Parasite Concentrator and evaluated by Optical Microscopy (OM). The “cellophane tape test” can be used for *E. vermicularis* identification. Some confirmation cases were provided by immunochromatographic (IC) kits, such *E. hystolitica/dispar* (RIDA®QUIK Entamoeba), *Giardia* and *Cryptosporidium* (RIDA®QUICK Cryptosporidium/Giardia Combi).

Results/Discussion: The total of 6932 faecal samples were collected from 2615 pacients, 356 samples were positive for IP. IP results were: female gender 55% against 45% for male gender. The mean age was 65 and 45 years respectively (p=0.012). There was a main prevalence for IP from 30-39 years (18.0%) followed by 50-59 years (16.1%), then 40-49 years (14.4%) and from age 3-10 years (12.1%). There wasn´t an association betwen gender and parasite (p> 0,05).The prevalence of parasites on IP was: *E. hartmanni* (49.3%), *Blastocystis sp.* (23.0%), *E. coli* (14.6%), *G. lamblia* (7.3%), *E. vermicularis* (2.5%), *E. nana* (2.0%), *A. lumbricoides* (0.6%), *Cryptosporidium* (0.6%), *E. hystolitica/díspar* (0.3%). Some yeast cells were identified in 389 samples but an egg, quist or parasite was rarely found. For all patients ≤ 2 years a IC test was performed to screen for *Cryptosporidium*. On this study, the number of samples was a little higher at the fourth trimester for both years, without a specific season peak. A higher positivity to *E. vermicularis* was expected, eventually the clinical request or the sample collect was not efficient. There are new factors that could influence the IP: new eating habits, sociodemographic factors, a "pet friendly"country and institutionalized psychiatric patients and immunosuppressed ones. The study shows that: female gender had the major prevalence; there was a low prevalence for IP comparing to other areas from the world. A correct collected sample with the best orientations, is fundamental for the laboratory performance and study.







P88

THE IMPORTANCE OF MEASURING CALCIUM LEVELS IN ONCOLOGICAL DISEASE

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Introduction: Hypercalcemia of malignancy (HCM) affects 2 to 30% of patients with cancer and is associated with high morbidity and mortality.

HCM causes can split into different categories, including: local osteolytic hypercalcemia, humoral hypercalcemia malignancy; 1,25(OH)2D-secreting lymphomas and ectopic hyperparathyroidism.

It occurs more often in patients with solid tumors, particularly in breast cancer, kidney cancer and hematological malignancies. Classified as: Mild: SCa 10.5 to 11.9 mg/dL [<3 mmol/L]; Moderate: SCa 12.0 to 13.9 mg/dL [3-3.5mmol/L]; Severe: SCa > 14 mg/dL [>3.5 mmol/L]. Clinical manifestations of HCM are variable and depend on the severity and rate of its development. HCM can affect the cardiovascular, gastrointestinal, renal, neurological, and musculoskeletal systems. The treatment will depend on the cause of the high blood calcium. However, there are several medicines that can help lower blood calcium, regardless of the cause, including: bisphosphonate, denosumab, hydration and calcitonin.

This clinical case aims to exemplify hypercalcemia associated with cancer.

Clinical Case: Female patient, 43 years old, diagnosed with bilateral invasive lobular mammary carcinoma with left axillar lymph node and liver metastization. Started palliative chemotherapy: weekly Paclitaxel + double lock. Levels of calcium increased during the treatment (**SCa** 14.3 ® 11.3 ® 23.0 mg/dL; **iCa** 1.84 ® 1.24 ® 1.46 ® 2.59 mmol/L), and at the last appointment for chemotherapy, severe hypercalcemia was documented, with compatible symptoms (altered state of consciousness, renal and hepatic failure). Given these results, bone metastases were suspected, even though the bone scintigraphy performed in 13/12/23 did not show any alterations. She was hospitalized on 04/03/24 and was given a hypocalcemic therapy: IV saline hydration, furosemide and zoledronic acid. Tumor markers also increased during treatment, which shows the unfavorable evolution of the disease.

Discussion: Hypercalcemia associated with malignancy is highly common, particularly at advanced stages of the disease. Given that the condition can progress to renal failure, coma and death, it is key to detect hypercalcemia to establish the treatment on time. Besides, measuring the levels of calcium is an accessible and affordable test.

P89

UNEXPECTED BLOOD ABNORMALITIES FOLLOWING SEVERE TRAUMATIC BONE INJURIES SUGGESTING LIPID DROPLETS IN CIRCULATION?

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Introduction: To ensure the clinical laboratory promotes the highest quality standards, it's crucial that the analytical process complies with the highest principles of technical accuracy. Laboratory professionals are expected to recognize unreliable results, identify the potential causes, and be acquainted with the ways to obtain accurate results. In this context, we present a case study detailing the atypical presence of fat droplets in peripheral blood after a severe trauma.

Case Report: A 39-year-old man was admitted to the emergency department with several bone fractures after a high energy trauma. The initial laboratory study revealed hemolysis of the sample, and an abnormal WDF (SSC-FSC) scattergram obtained on the hematology analyzer Sysmex XN-20. The macroscopic appearance of the blood smear was abnormal. Under microscopic scrutiny, numerous voids were observed, ranging from circular to oval shapes of diverse dimensions, likely

indicating the presence of particles of lipidic composition that dissolved during the staining process. Visual inspection of the serum revealed mild hemolysis but no turbidity. After centrifugation of the sample obtained in the EDTA-K3 tube, we observed a whitish-yellowish layer within the supernatant. It is noteworthy that the patient was not receiving intravenous lipids, and the abnormalities described were not present in the subsequent sample.

Discussion: Altogether, the laboratory findings suggest the presence of fat droplets in circulation. It is important to consider it in the proper clinical context, especially in cases involving bone trauma, intravenous administration of lipid emulsions or contamination of the blood sample with subcutaneous adipose tissue during traumatic venipuncture. The occurrence of fat droplets is a rare phenomenon that may interfere with the analytical process, namely the white blood cell count and differential, depending on the technology applied. In the present report we did not find relevant interference with the results obtained via Sysmex analyzers although the scattergrams clearly identified an altered distribution of events. Laboratory professionals should be aware of such interferences, enabling prompt recognition and appropriate action.

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AN OVERVIEW OF K ANTIVITAMINS IN CURRENT CLINICAL PRACTICE

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Introduction: The contemporary history of the vitamin K antagonists (VKAs) began more than a century ago with the discovery of the first oral anticoagulants, such as dicumarol, in North America. Until a few years ago, VKAs were the only oral anticoagulants available. The use of VKAs has declined with the introduction of direct oral anticoagulants (DOACs), but they are still widely prescribed. The main objective of this study was to review the VKAs drugs available in current clinical practice, focusing on the VKAs available drugs in Portugal and their main indications today.

Methods: We conducted a review study based on a bibliographic search of articles collected from Google Scholar, MEDLINE and PubMed databases and reference books.

Results and discussion: Warfarin, a coumarin derivative, was approved for clinical use in 1954. Subsequently, two other coumarin derivatives were developed and introduced for clinical use in Europe: phenprocoumon and acenocoumarol. Warfarin is the most widely used in the world. Acenocoumarol and phenprocoumon are second in use in several continental European countries, but they are not available in the United States. Dicumarol and indanedione derived VKAs (*fluindione*, phenindione, others) are not widely used, with the exception of *fluindione* in Luxembourg, Switzerland, and also France where it has been the most prescribed VKA for decades. In Portugal, warfarin is the most widely used, followed by acenocoumarol, and no indanedione derivative is available. These drugs have essentially the same properties, but differ in onset, duration, intensity of action, adverse reactions and indications. VKAs remain the gold standard of oral anticoagulant therapy for the secondary prevention of thromboembolic events in patients with mechanical heart valves and in end-stage chronic kidney disease, and warfarin in other conditions requiring anticoagulation (thrombophilia, thrombosis of atypical location, others).

Conclusion: VKAs have been the mainstay of anticoagulation therapy for more than 60 years, and their use remains current. In recent years, the knowledge of these drugs seems to be declining, especially among younger physicians. It is therefore important to continue training in their prescribing, as the management of VKAs is difficult.

Keywords: Oral Anticoagulation, VKAs, DOACs

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MULTIPLE MYELOMA: A CASE STUDY WITH MINOR LABORATORY MANIFESTATIONS

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Introduction: Multiple myeloma (MM) is a malignant monoclonal gammopathy which accounts for 1% of all cancers and 10-15% of haematological malignancies. It has a globally variable incidence and 95% of cases occur in patients aged > 40 years. MM is a plasma cell neoplasm characterized by the clonal proliferation of plasma cells in the bone marrow usually producing a monoclonal immunoglobulin (detected in serum and/or urine) and related organic damage: hypercalcemia, renal failure, anaemia and bone lesions (CRAB). The clinical disease is preceded by pre-oncological phases, namely monoclonal gammopathy of undetermined significance (MGUS) and smouldering myeloma. The vast majority of MM produce a complete clonal immunoglobulin or free light chain, rarely, 1-2% are oligo or non-secretory.

Clinical Case Report: A 47-year-old Caucasian man under study at hospital consultation after detection of multiple lytic bone lesions on X ray while studying a refractory dorsal pain with a 3-month evolution was referenced to the emergency department due to paraparesis grade 4/5 and spastic paraparetic gait. Dorsal and lumbar spine computed tomography (1-2-24) revealed multiple lytic lesions and a dorsal epidural mass (probable hematologic neoplasm) with dorsal spinal cord compression. The patient underwent an urgent magnetic resonance imaging followed by surgery with partial excision of the lesion and spinal cord decompression. Serum and urinary protein electrophoresis did not show any M spike. He presented an abnormal free light chain (FLC) ratio of 2.3 [0,26 - 1,65]. Blood tests prior to surgery (1-2-24) without significant abnormalities (haemoglobin, creatinine, total proteins, calcium and beta-2 microglobulin). The bone marrow aspirate (19-2-24) showed 12% of clonal plasma cells with an aberrant immune phenotype.

Conclusion: This case illustrates an oligo secretory MM with few laboratory abnormalities. It emphasizes the need to consider MM in patients with persistent clinical symptoms even in the absence of classical laboratory abnormalities. MM diagnostic procedures involve a combination of laboratory, imaging and histological tests in order to confirm the presence of plasma cell disorders in need of therapeutic intervention.