17ª Reunião Científica da Sociedade Portuguesa de Medicina Laboratorial

03 a 05
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Centro de Congressos
do Porto Palácio Hotel

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CO01

ESTABLISHING REFERENCE INTERVALS FOR WHITE BLOOD CELL PARAMETERS IN PORTUGUESE NEWBORNS

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Objectives: This research aims to determine RIs for hematological parameters, with a particular focus on white blood cells (WBC), in Portuguese newborns. An indirect approach utilizing secondary data will be employed, following the guidelines of the International Federation of Clinical Chemistry and Laboratory Medicine.

Methods: A total of 4,771 hemogram results from newborns aged 0 to 7 days were extracted from the hospital laboratory information system, covering tests performed between 2013 and 2019. These results were obtained from venous blood samples collected in K3EDTA (Sarstedt) 1.1 mL tubes and analyzed using the Sysmex XE-5000 hematology analyzer. Exclusion criteria included preterm newborns, newborns with more than one result, elevated bilirubin levels, and elevated C-reactive protein levels. Outliers were successively removed using Tukey's method, with the process repeated if necessary. After all exclusions, 1,254 hemograms were selected, and RIs were calculated using the non-parametric percentile method, defined as the 2.5th and 97.5th percentiles. The parameters included in this study were white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MON), eosinophils (EOS), and basophils (BASO), presented in both absolute values and percentages for differential counts.

Results: The RIs determined were as follows: WBC 10.40-28.74 EXP3/uL, NEUT 49.00-81.00 % and 5.78-21.35 EXP3/uL, LYMPH 12.00-42.00 % and 2.16-8.18 EXP3/ul, MON 1.51-12.40 % and 0.22-2.79 EXP3/uL, EOS 0.00-5.5 % and 0.00-1.06 EXP3/uL, and BASO 0.00-0.5 % and 0.00-1.06 EXP3/uL.

Discussion: Studies on RIs in newborns are scarce. Our results for total WBC and NEUT counts appear to be higher than those reported in most sources;
However, these studies often include a broader age range in their first stratification. In contrast, our findings are more consistent with the limited studies available that specifically focus on newborns within the first 24 hours of life.

Conclusions: This study establishes, for the first time, WBC RIs for newborns in a Portuguese population, providing valuable insights at local, national, and international levels due to the limited number of studies in this field.
The availability of these RIs is particularly relevant, as they play a crucial role in clinical decision-making.

CO02

CHIMERISM AFTER SECOND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A CASE REPORT

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Introduction: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an increasingly relevant therapeutic option for the treatment of hematologic diseases, as it replaces the patient's hematopoietic cells with normal cells from the donor. Chimerism (CM) analysis post-allo-HSCT assesses the proportion of donor and recipient hematologic cells, serving as a key predictor of transplant success. This technique relies on genetic markers—highly polymorphic microsatellites (STRs)—to differentiate donor and recipient alleles. In a pre-HSCT diploid situation, each locus contains one allele from the mother and one from the father, presenting as two peaks (heterozygosity), or if they coincide, only one peak (homozygosity). Using this methodology, the analysis and interpretation of the electropherograms allow the identification of the origin of the studied alleles (donor and/or recipient), as well as their relative quantification.
This case presentation aims to demonstrate that, even in complex and challenging scenarios, chimerism remains a valuable tool for evaluating patients after allo-HSCT.

Case Description: A 53-year-old woman diagnosed with multiple myeloma in 1994 underwent allo-HSCT from an HLA-identical sister, achieving remission. After relapsing in 1999, she received an autologous bone marrow transplant and remained in remission. In November 2022, she was diagnosed with myelodysplastic syndrome and is scheduled for a second allo-HSCT from an HLA-compatible brother in July 2024 at IPO-Porto. She is currently under follow-up with complete chimerism. In related HSCT, shared alleles are more likely, affecting chimerism analysis, which relies on donor-recipient allelic differences. Successful HSCT results in a donor-only allelic profile.

Discussion: Thirty years after her 1st allo-HSCT, a pre-2nd HSCT study revealed that the patient's electropherogram displayed 2, 3, or 4 alleles per locus, with unclear origins (recipient or 1st donor). Given that both donors were related, the number of informative markers was limited to 3. These markers showed 2 alleles per locus: 1 shared between the pre-2nd HSCT patient and the 2nd donor, and another distinct allele, confirming its origin from the 2nd donor.
The application of this technique demonstrated that, despite the complexity of the case—where the patient underwent two related allo-HSCT procedures—chimerism could be effectively evaluated.

CO03

REACTIVE PERIPHERAL BLOOD PLASMACYTOSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE

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Background: Reactive plasmacytosis is defined as an abnormal increase in polyclonal plasma cells, either in bone marrow and/or peripheral blood, secondary to a pathophysiological process. Likely causes include infections (32%), angioimmunoblastic T-cell lymphoma (23%), drugs (19%), malignancies (12%), miscellaneous disorders (8%), and autoimmune diseases (6%). [1] Herein, we report a case of reactive peripheral blood plasmacytosis in a patient with systemic lupus erythematous (SLE), whose laboratory diagnostic workup was handled by the Clinical Pathology Department.

Case presentation: An 81-year-old woman with SLE and no further medical history of relevance presented to our hospital for a follow-up appointment within the Rheumatology Department. A complete blood cell count was performed as part of routine evaluation. The automated haematology analyser revealed an unexpected monocytosis (32%; 2.4×109/L), with abnormal monocytes scatter and blasts alarm in monocytes region. Analysis of peripheral blood smear using light microscope and digital morphology analyser showed normal monocytes count (9%; 0.7×109/L), but enhanced plasma cells (15%; 1.1×109/L) which have been misclassified as monocytes by the automated analyser. Patient presented 12.4 g/dL haemoglobin, 49 mm/h sedimentation rate and 53 mL/min/1,73m2 glomerular filtration rate, without other analytical alterations. Given the concerns of a plasma cell neoplasm, our department took the initiative to pursue further investigations for clonality assessment. Flow cytometry of peripheral blood found 31% polyclonal plasma cells without phenotypic anomalies (K:L ratio 2.1:1; CD138+, CD38+, CD19+, CD45+, CD56–). Serum protein electrophoresis revealed a slight polyclonal hypergammaglobulinemia (24%; 18.1 g/L). Serum immunofixation did not find monoclonal immunoglobulins. Therefore, a reactive plasmacytosis secondary to SLE was admitted, avoiding reference to a haemato-oncology appointment.

Conclusions: Reactive plasmacytosis poses a diagnostic challenge as it may mimic plasma cell neoplasms. Thus, comprehensive investigations to discern the underlying cause of high plasma cells count must be undertaken.

In this frame, the laboratory may have a privileged role by offering a collaborative workup through anticipating the clinicians' needs, particularly through immunophenotyping.

Ultimately, this may prevent additional medical appointments.

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CO04

ORAL ANTICOAGULANT THERAPY IN THROMBOPHILIA: CURRENT EVIDENCE AND CLINICAL CHALLENGES

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Introduction: Thrombophilia is a hereditary or acquired condition that increases the risk of venous thromboembolism and arterial thrombosis, often requiring long-term anticoagulation.
The choice between vitamin K antagonists (VKAs), such as warfarin, and the more convenient direct oral anticoagulants (DOACs) remains controversial, particularly in acquired thrombophilia such as the antiphospholipid syndrome (APS).

Objective: To compare the efficacy and safety of VKAs and DOACs in hereditary and acquired thrombophilia.

Materials and Methods: A review of the literature was conducted, including randomised clinical trials, meta-analyses, and international guidelines. Thromboembolism recurrence rates and bleeding events were assessed in low-risk thrombophilias, such as Factor V Leiden (FVL) and prothrombin (PT) mutations, and in high-risk conditions, including APS, protein C (PC), protein S (PS), and antithrombin (AT) deficiencies.

Results: In low-risk thrombophilias, including FVL and PT mutations, DOACs have shown similar efficacy and safety when compared to VKAs, making them a potential alternative. However, the evidence for DOACs in rare thrombophilias, such as PC, PS, and AT deficiencies, remains limited, and there is no clear consensus on their use. In APS, warfarin remains the standard treatment, particularly in patients with triple positivity for antiphospholipid antibodies (aPL). A meta-analysis of randomised controlled trials comparing DOACs versus warfarin in APS patients showed a significantly increased risk of recurrent arterial thrombosis, despite no evidence of a higher risk of recurrent venous thromboembolism or bleeding.
Accordingly, current international guidelines recommend an individualised approach, taking into account the patient's thrombotic risk profile, history of thrombotic events and treatment adherence when selecting an anticoagulant drug.

Conclusions: DOACs have been shown to be a viable and effective alternative to VKAs in low-risk thrombophilias.
However, they are less effective in preventing thrombosis in APS, which is particularly concerning due to the risk of arterial thrombotic events.
Further randomised trials are needed to clarify the role of DOACs in rare thrombophilias and specific APS subgroups to ensure an evidence-based, patient-centred anticoagulation strategy.

CO05

ACUTE BIPHENOTYPIC BLAST CRISIS: A RARE CONDITION CASE REPORT

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Introduction: Chronic Myeloid Leukaemia (CML) is a myeloproliferative disorder characterized by the uncontrolled growth of myeloid cells. The disease generally progresses through 3 stages: a chronic phase, followed by an accelerated phase and eventually a terminal blast crisis. A blast

crisis is defined as the presence of more than 20% blasts in the peripheral blood smear (PBS) or bone marrow (BM). The blasts may be classified as myeloid, leading to acute myeloblastic leukaemia, or lymphoid, which results in acute lymphoblastic leukaemia (ALL).
In rare cases, the blast crisis may present with biphenotypic expression for both lineages. In these instances, the disease may progress more aggressively and is associated with a poorer prognosis, particularly if there is involvement outside the bone marrow.

Case Report: A 65-year-old man was sent to the emergency care with aspiration pneumonia. Laboratory studies revealed elevated white blood cell count (18500/uL), anaemia (Hb 8.0 g/dL) and low platelet count (37000/uL). Pathologic review of PBS showed remarkable leukocytosis and 43% blasts with characteristics of both myeloid and lymphoid lineages.
The patient had a known history of CML diagnosed 4 years prior to presentation, in which chromosomal analysis revealed BCR-ABL1 fusion. Phenotyping was difficult to characterize due to the coexistence of biphenotypic immunohistochemical markers. For unknown reasons, a BM aspirate was not carried out.
Given these clinical findings, the diagnosis of transformation of CML to Acute Biphenotypic Leukaemia (ABL) was established.

Discussion: The development of acute biphenotypic leukemia (ABL) is believed to be derived from BM stem cells that have the ability to express antigens from more than one cell line. The incidence of ABL in adults varies between 1% and 20% of acute leukaemias. The t(9,22)(q34;q11) is the most common cytogenetic anomaly. Since mortality rates are close to 100% despite aggressive chemotherapy, the prognosis is poor when compared to de novo AML or ALL. There are no defined diagnostic criteria for ABL, which highlights the importance of combining all laboratory and clinical findings. Case reports such as this one end up being an important source of education, promoting discussion and better patient care.

CO06

ACQUIRED HEMOPHILIA A: CASE REPORT AND LABORATORY DIAGNOSIS

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Introduction: Acquired hemophilia A is a rare autoimmune coagulopathy caused by autoantibodies against endogenous factor VIII. Clinically characterized by spontaneous hematomas, while hemarthrosis is uncommon, and primarily affects elderly individuals.
While its etiology remains unclear in most cases, it can be linked to pregnancy, autoimmune diseases, malignancies, infections or drug-induced mechanisms.
Diagnosis relies on clinical presentation, prolonged activated partial thromboplastin time (aPTT) and significantly reduced factor VIII levels.

Case Description: An 83-year-old woman presented to the Emergency Department (ED) with spontaneous hematomas on her left arm and right leg, not related with trauma or anticoagulant use. Radiological exams showed no fractures, and an arthrocentesis of the right knee revealed hemorrhagic effusion. The patient was initially discharged but returned five days later with worsening ecchymoses on the left hemiface and costal region. Laboratory tests showed severe macrocytic anemia (4.9 mg/dL), normal platelet count and significantly prolonged aPTT (83.20 sec). Factor VIII levels were undetectable (0%), while factors IX (56.4%) and XI (55.4%) were preserved and lupus anticoagulant testing was negative. A two hour mixing test at 37°C partially

corrected aPTT (44.7%), though not to reference values, suggesting the presence of a factor VIII inhibitor, confirmed by a Bethesda assay, that revealed an inhibitor title of 15 UB.
The patient was treated with transfusional support, prednisolone (20 mg), and activated coagulation proteins (1000 U/day), leading to a progressive reduction in aPTT while maintaining low factor VIII levels.
After 20 days of hospitalization, with stabilized hemoglobin (10.4 mg/dL) and reduced inhibitor potency (2.6 UB), the patient was discharged with prolonged aPTT and low factor VIII levels, continuing corticosteroid therapy and referred to immunohematology for follow up.

Discussion: The low prevalence of acquired hemophilia A (1.5 cases per million) delays diagnosis and intervention.
Rapid laboratory assessment is essential since these specific tests are not part of routine coagulation testing.
In conclusion, early suspicion and a personalized treatment approach are crucial in the management of acquired hemophilia A, preventing severe hemorrhages, and improving outcome.

CO07

PSEUDOTHROMBOCYTOPENIA, IS VORTEXING THE WAY OUT?

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Introduction: Pseudothrombocytopenia (PTP) leads to incorrect diagnoses, unnecessary treatments, and repeated blood tests. This in vitro phenomenon is well-documented with methods proposed to address it.
The use of vigorous mechanical agitation (vortexing) of EDTA tube samples for 1-2 minutes stands out. Specific guidelines for managing PTP in routine lab tests are still needed.

Objective: Establish a standardized internal protocol to reduce PTP by normalizing PLT counts through a vortexing protocol.

Materials and Methods: This study included blood samples in EDTA tubes from patients with complete blood count (CBC) requests. Upon confirmation of PLT aggregates through microscopic observation of the blood smear, the samples were vortexed at maximum power for 2 minutes. Samples containing fibrin were excluded.
The CBC was repeated in the automatic analyzer within 2 to 3 minutes, and a new blood smear was prepared.
For requests that included samples collected in sodium citrate tubes, PLT counts were also performed and adjusted by multiplying by 1.1 to account for the different blood-to-additive ratios.
Several CBC laboratory parameters were evaluated: PLT count, mean PLT volume (MPV), plateletcrit (PCT), PLT distribution width (PDW), hemoglobin (HGB), red blood cell count (RBC), white blood cell count (WBC), and the presence of aggregates in the blood smear.
The automated analyses were performed on the Sysmex XN 2000™. Microsoft Excel (Version 16.93.1) was used for data treatment and statistical analysis.

Results: The parameter analysis revealed the following variations:

	WBC (103/µL)	RBC (106/µL)	HGB (g/dL)	PDW (fL)	MPV (fL)	PCT (%)	PLT (103/µL)
Mean	0,14	0,02	0,10	-0,35	-0,13	0,10	84.73
SD	0,30	0,06	0,19	3,06	0,86	0,08	67.77

Out of 68 samples with initial PLT counts below 100x103/µL, 47 surpassed this threshold after treatment, achieving a normalization rate of 69.12 %. An analysis of 19 samples collected in sodium citrate tubes revealed an average difference in PLT counts of 16.29x103/µL (SD 45.7x103/µL) when compared to the respective samples collected in EDTA post-procedure.

Conclusion: By applying this laboratory technique, it is possible to achieve normalization of PLT counts in a large percentage of cases, without significant changes in other blood count parameters. Thus, avoiding repeated blood tests. The presented technique offers a straightforward, rapid, and easily implementable procedure.

CO08

PERITONEAL DIALYSIS AND PERITONEAL FLUID ANALYSIS

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Introduction: Peritoneal dialysis is an important renal replacement therapy for children with end-stage renal disease.
Fungal peritonitis represents 3 to 6% of all cases of peritonitis in patients undergoing peritoneal dialysis, [1] and is associated with serious morbidity, mortality, and technique failure. [1, 2, 3] Candida peritonitis can be responsible for invasive candidiasis and, consequently, the increase mortality rate. [1, 2] C. albicans is the main pathogen in Candida peritonitis [1, 2], but C. parapsilosis is responsible for more complications and worse prognosis than other Candida species in peritoneal dialysis fungal peritonitis. [1]

Case description: A 14-year-old boy with chronic kidney disease (stage 5) on automated peritoneal dialysis is admitted to the emergency room with abdominal pain and fatigue for three days and the presence of cloudy peritoneal fluid. The patient reports no fever, vomiting or nausea. Analytically he presents hemoglobin 121 g/L (reference value (RV): 13-17.5 g/L)), leukocytes 11.3×109/L (RV: 4-11×109/L), neutrophils 8.23×109/L (RV: 1.9-7.5×109/L), C-reactive protein 841.9 nmol/L (RV:<47.62 nmol/L), and creatinine 0.93 mmol/L (RV: 0.04-0.07 mmol/L).
A sample of peritoneal fluid sent to the laboratory for cytological analysis showed the presence of 9000 cells/µL.
Microscopic examination of the peritoneal fluid showed the presence of neutrophils (70% of total cells (TC)), lymphocytes (29% of TC), eosinophils (1% of TC), and the presence of spherical to ovoid yeast cells within the neutrophils (sometimes destroyed) and outside. This finding led to the addition of an antifungal to therapy. Two days later colonies of Candida parapsilosis developed in the culture media.

Discussion: The best approach to improve patients' survival is prompt diagnosis of fungal peritonitis.[3]
Therefore, early diagnosis and management are essential.
This case highlights the significant role that cytological analysis can play in the early diagnosis and treatment of fungal peritonitis.

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CO09

A CASE OF SEVERE HYPERVISCOSITY SYNDROME AS INITIAL PRESENTATION OF MULTIPLE MYELOMA

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Introduction: Multiple myeloma (MM) is a haematological lymphoid malignancy with plasma cell proliferation in the bone marrow and increased production of monoclonal immunoglobulins. Most cases evolve from an asymptomatic stage of either monoclonal gammopathy of undetermined significance or smouldering myeloma. Patients with symptomatic disease usually present with fatigue, weight loss and anaemia.
Clonal bone marrow plasma cells ≥10% or plasmocytoma and one or more myeloma defining events (hypercalcaemia, renal insufficiency, anaemia or bone lesions) confirm the diagnosis.

Clinical Case: A 66-year-old male, obese, hypertensive, former smoker, on quadruple treatment presented to the Emergency Department with a history of nausea and three episodes of syncope. The blood pressure was 140/103 mmHg with a heart rate of 118 beats per minute, the ECG showed sinus rhythm with inferior wall Q waves and slight ST segment depression from V2 to V6. A basic laboratory analytic panel was requested with cardiac markers.
The full blood count showed anaemia (11.2 g/dL). Only a few chemistry parameters were determined, due to incomplete separation of the serum and gel. A new sample was collected without gel. Total proteins were 140.8 g/L.
The clinical team was alerted and laboratory tests for myeloma were also added. When separating the sample for further processing, it presented with a solidified gel-like appearance. Only after prolonged, high-speed centrifugation (approximately 5000 g for 10 minutes) was it possible to process the sample.
The serum protein electrophoresis revealed a sharp peak in the gamma globulin region, serum IgG was 7770 mg/dL, with a free-kappa-lambda ratio of 14.72.
Adequate treatment with bortezomib, dexamethasone and thalidomide was promptly initiated.

Discussion: We report a rare case of IgG MM presenting with hyperviscosity syndrome (HVS). HVS is an infrequent complication of MM (mainly IgM) presenting with mucosal bleeding, visual or neurological manifestations. It is a medical emergency with a high mortality rate. Such an elevated protein level might cause technical difficulties in sample processing. In this case, these difficulties were the first cue towards a correct diagnosis.

CO10

ACQUIRED HEMOPHILIA A FOLLOWING A BLEEDING DISORDER - CASE REPORT

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Introduction: Hemophilia A and B are congenital bleeding disorders caused by a deficiency or complete absence of coagulation factor VIII (FVIII) or factor IX (FIX), respectively.
These X-linked disorders represent the large majority of inherited deficiencies of clotting factors, occurring in approximately one per 5000 and one per 50,000 male births, with no racial predilection. Individuals with a factor level 1 IU/dL are classified as severe hemophiliacs and represent about half of diagnosed cases.

Case description: A 73-years-old male, admitted to the Emergence Room due to edema of the knee and left thigh with pain on mobilization, with no history of trauma. In MCDT: Leukocytosis (23.1 g/L), with neutrophil (19.77 g/L), normo/normo anemia (Hb 9.0 g/dL), increase in aPTT (63.2 sec), worsening of renal function (Urea 117 mg/dL, creatinine 2.9 mg/dL). He has been operated on and received a total of 14 red cell units and 8 fresh frozen plasma units. One month later, he developed fever. Laboratory tests revealed a prolonged aPTT and a high Bethesda assay, indicating acquired haemophilia A with factor VIII deficiency [Factor VIII, Specific Inhibitors (residual activity - 29% - low; Bethesda Units – 719UB/mL - high)]; PT202100A mutation – normal; FV Leiden mutation – normal; FVIII - undosable (low); FIXc – 23% - low; FX – normal; FXI - 25%; FXII - 24%; FiXc - 55%; Antithrombin III - 101.0%; Functional protein C - 115%; Free Protein S - 79.3%; FvW Ag - 414.2%; Ac. anti-Xa - <0.04 IU/ml; Lupus anticoagulant – negative. In acquired hemophilia A, there is the production of antibodies known as coagulation inhibitors, mainly against factor VIII, leading to the appearance of bleeding and alteration of the aPTT.

Treatment: prednisolone; rituximab (is a genetically engineered mouse/human chimeric monoclonal antibody representing a human IgG1 glycosylated immunoglobulin with constant regions and variable sequences from mouse light and heavy chain regions); FVIIIa until bleeding control.

Discussion and conclusion: Acquired hemophilia A is an uncommon but severe bleeding disorder. It is caused by the development of autoantibodies directed against one of the antihemophilic factors, most frequently factor VIII (FVIII). The diagnosis of this disease is established with difficulty because of its rarity and the complexity of the laboratory diagnosis. It must be diagnosed and treated promptly to avoid the high morbidity and mortality associated with this condition.

CO11

CARCINOMA CELLS IN PLEURAL FLUIDS – A CHALLENGE

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Introduction: The identification in hematology laboratories (HL) of neoplastic cells in biological fluids can become a challenge.
They are usually present in low proportions, the cytocentrifugation process leads to distortion of cells morphology, neoplastic cells can sometimes have the same appearance as normal cells (for example, making it difficult to distinguish between normal or reactive mesothelial cells and

neoplastic cells) and the staining techniques available in HL are limited. The last point can only be overcome with the use of specific techniques used only in histopathology laboratories.

Cases description: Four pleural fluid samples from four different patients were received and processed in the hematology laboratory.
The smears were stained with May-Grunwald-Giemsa stain.
Later and after microscopic observation, it was possible to identify carcinoma cells, with different morphological characteristics in each sample, highlighting the difficulty in the characterization and identification of these cells.
Making this procedure a major challenge in the hematology laboratory. Generally, they are medium to large cells with an immature chromatin nucleus with or without visible nucleoli and intensely basophilic cytoplasm with, sometimes, polar vacuoles. Sometimes these cells aggregate together forming a cellular nest in which a cellular syncytium can be seen. All of these samples were later processed in the histopathology laboratory (Laboratório de Anatomia Patológica) confirming the initial suspicion

Discussion: This manuscript highlights the important role that the hematology laboratory can have in the recognition of specific cellular characteristics helping in the early identification of metastatic cells, allowing for timely diagnosis and treatment of this condition.

CO12

AMR FINDINGS IN A MOLECULAR BIOLOGY LABORATORY BY REAL-TIME PCR ASSAY DURING THE YEAR 2024

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Background: The global spread of antimicrobial-resistance (AMR) is an increasing public health concern, threatening the advancements of modern medical care over the past century.
The emergence and spread of carbapenemase-producing Enterobacteriaceae (CPE), Extended Spectrum Beta-Lactamase-producing Enterobacteriaceae (ESBL-E), and vancomycin-resistant Enterococci (VRE) in healthcare environments are often difficult to treat, leading to higher morbidity and mortality rates as well as increased healthcare costs.
Conventional methods for detecting AMR in clinical samples have the drawback of requiring 2 to 3 days after sample collection.
In contrast, real-time polymerase chain reaction (RT-PCR) based assays can quickly detect multiple genetic resistance determinants, regardless of bacterial species, making them essential tools for diagnostic and clinical decision-making.

Aim: To present a prospective diagnostic and epidemiological study of CPE, ESBL-E, and VRE from rectal swabs of patients in a molecular biology laboratory during the year 2024, using the Allplex™ Entero-DR Assay.

Methods: The Allplex™ Entero-DR Assay is a qualitative in vitro diagnostic test that uses real-time multiplex PCR for the detection of five carbapenemase-encoding genes (blaKPC, blaVIM, blaIMP, blaNDM, blaOXA-48-like), one ESBL gene (blaCTX-M), and two vancomycin resistance markers (vanA and vanB), all in a single PCR reaction in approximately 3 hours.

Results: A total of 2082 clinical rectal swabs were analysed and considered valid. Approximately 66% (1377/2082) of the samples were negative.
All CPE genes were detected being blaKPC the most prevalent, appearing in 80% (62/78) of the positive CPE samples. The blaCTX-M appeared in almost 20% (407/2082) of the sampling and 17% (353/2082) presented vancomycin resistance markers, of which 96% (340/353) were vanB positive.

Conclusion: This study accentuates the importance of rapid diagnostic and infection control practices.
It is in agreement with studies that showed blaKPC as the most prevalent among CPE genes, highlights the worrying increase of ESBL-E and supports that VRE has shifted from a predominance of vanA over the years towards vanB. Allplex™ Entero-DR Assay enables rapid and efficient identification of multiple resistance markers and is a highly accurate test with significant lower turnaround time (TAT) and may, therefore, be regarded as a reliable and fast epidemiological tool.

CO13

UNVEILING THE PREVALENCE OF SEXUALLY TRANSMITTED INFECTIONS: INSIGHTS FROM MULTIPLEX RT-PCR AND THE ROLE OF PREP CONSULTATIONS

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Sexually Transmitted Infections (STIs) represent a significant global public health challenge. More than 1 million people acquire an STI every day, and it is estimated that approximately 500 million people worldwide contract one of four major STIs: chlamydia, gonorrhea, syphilis and trichomoniasis. Some STIs can increase the risk of acquiring HIV by threefold or more. Within the scope of the Priority Program for sexually transmitted infections and HIV infection, access to HIV Pre-Exposure Prophylaxis (PrEP) for individuals at increased risk of infection is a key priority, as it is a highly effective prevention strategy in reducing the incidence of new HIV cases.

The primary objective of this study was to identify the predominant STIs in our region in 2024, particularly in individuals attending PreP consultations. A total of 723 samples were collected, and pathogen detection was performed using real-time PCR with the Allplex™ STI Essential Assay (Seegene).

STI-causing pathogens were detected in 401 samples (55,5%). Ureaplasma parvum (UP) was the most prevalent (35,4%), followed by Ureaplasma urealyticum (UU) (21,7%) and Mycoplasma hominis (MH) (19,7%). Less common were Neisseria gonorrhoeae (NG) (9,5%), Chlamydia trachomatis (CT) (9,0%), Mycoplasma genitalium (MG) (3,0%) and Trichomonas vaginalis (TV) (1,7%). Young adults (19-40 years old) had the highest STI prevalence (64,3%). Although more male samples were tested (n=425), STIs were more frequently detected in female samples (56,6%).
Among samples from PrEP consultations (26,8%of the total), STI-causing agents were identified in 30,4% of cases. UU (39,0%), MH (23,7%) and NG (15,2%) were the most prevalent. Unlike

other consultations, UP was the least prevalent, representing 5,1% of positive samples. Notably, 96,3% of the samples from PrEP consultations were from young adult males.
These findings highlight the significant prevalence of STIs in our region, with some differences between general consultations and PrEP users. The high detection rates of UU and MH in PrEP consultations suggest the need for further investigation into their clinical relevance in this population.

Additionally, the increased prevalence of NG reinforces the importance of targeted screening and prevention strategies. Strengthening STI surveillance and enhancing education on safe sexual practices may contribute to improved infection control and better public health outcomes.

CO14

GUT MICROBIOTA AND THYROID CANCER: THE DECREASE OF FAECALIBACTERIUM PRAUSNITZII AS A POTENTIAL BIOMARKER

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Introduction: Faecalibacterium prausnitzii is a butyrate-producing anaerobic bacterium with anti-inflammatory properties and a significant role in maintaining intestinal homeostasis. Changes in its abundance have been associated with various pathologies, including neoplasms, but its profile in patients with differentiated thyroid carcinoma (DTC) remains poorly explored.

Objective: To evaluate the relative abundance of F. prausnitzii in the gut microbiota of patients with DTC, comparing it with that of healthy volunteers.

Materials and Methods: This prospective study included 37 patients with DTC and 10 healthy volunteers as the control group. Faecal samples were collected for gut microbiota analysis using shotgun metagenomic sequencing. The relative abundance of F. prausnitzii was quantified, and differences between groups were assessed using the Wilcoxon test. Alpha diversity was analysed using the Chao1, Simpson, and Shannon indices, while beta diversity was evaluated based on Bray–Curtis and Jaccard metrics.

Results: The relative abundance of F. prausnitzii was significantly lower in patients with DTC compared to the control group ($p = 0.02$).
LEfSe analysis confirmed F. prausnitzii as one of the most enriched species in the control group, with an LDA score > 4 and $p < 0.001$.
No significant differences were observed in alpha diversity ($p > 0.4$) or beta diversity ($p > 0.3$) between the groups.

Conclusions: Patients with DTC exhibit gut dysbiosis, characterised by a significant reduction in the relative abundance of F. prausnitzii compared to healthy volunteers.
This finding suggests a potential role for this bacterium in the pathophysiology of DTC, highlighting the importance of the gut microbiota in modulating processes related to thyroid cancer.

CO15

A HIDDEN CAUSE OF NONGONOCOCCAL URETHRITIS IN MEN: A CASE REPORT

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Introduction: Although Corynebacterium glucuronolyticum (C. glucuronolyticum) is a known coloniser of the male genitourinary tract, it has been increasingly associated with nongonococcal urethritis (NGU) and prostatitis, particularly as an opportunistic pathogen.

Case description: A 47-year-old man with no significant medical history presented to the andrology outpatient clinic with a 2-year chief complaint of persistent urethral discomfort.
He reported constant urethral burning sensation, but no pain during urination or sexual activity. Urologic physical examination was unremarkable, except for a probable left epididymal cyst. Hormonal tests were also normal. Sperm culture revealed moderate growth of small, convex, white, non-haemolytic colonies, later identified by MALDI-TOF MS as C. glucuronolyticum.
Antimicrobial susceptibility testing (AST), performed using disk diffusion according to EUCAST standards, demonstrated resistance to benzylpenicillin, fluoroquinolones, and clindamycin, and susceptibility to tetracyclines and vancomycin.
All major pathogens causing sexually transmitted infections (STI) were excluded using a multiplex real-time PCR panel. Based on these findings, a diagnosis of C. glucuronolyticum NGU was established, and the patient was treated with a 14-day regimen of doxycycline.

Discussion: C. glucuronolyticum remains an underrecognised and underestimated cause of NGU in men. This case underscores the importance of considering this pathogen in the differential diagnosis of persistent urethritis. Reliable identification and AST are crucial for ensuring appropriate treatment and preventing potential complications.

CO16

MULTIPLEX PCR: REVOLUTIONIZING THE DIAGNOSIS OF INFECTIOUS GASTROINTESTINAL DISEASES

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Gastrointestinal disease due to infectious pathogens currently represents an important global health concern and is a major cause of morbidity and mortality worldwide, especially among young children and immunocompromised patients.
Diarrhea may result from infection with a variety of microbial pathogens, including bacteria, viruses or parasites. Historically, the diagnosis of infectious diarrhea has been made using microscopy, antigen tests and culture. Diagnosis has been revolutionized by the development of in vitro diagnostic (IVD) multiplex molecular panels for the detection of various pathogens nucleic acids.

A multicenter, cross-sectional study was conducted over a one-year period (2024). A total of 1547 samples were obtained from both outpatients and inpatients presenting with symptoms of acute gastroenteritis at three different healthcare facilities. The samples were then analysed using VIASURE® Gastrointestinal Panel I Real Time PCR Detection Kit. The results were analysed using IBM® SPSS® Software. The GI Panel positivity rate among 1547 samples was 33.6% (n =

520), of which 4.8% (n = 74) were co-infections. Bacterial agents were detected in 45.2% (235/520) of the samples, viral agents in 66.9% (348/520), and parasites in 3.8% (20/520).

Campylobacter was the most common bacterial pathogen, present in 78.7% (185/235) of bacterial-positive samples. Among viral agents, Norovirus GII was the most frequent, found in 29.9% (104/348) of samples, followed by Adenovirus at 26.72% (93/348). Cryptosporidium was the most prevalent parasite, identified in 55% (11/20) of parasitic infections. Gastrointestinal infections caused by Norovirus, Rotavirus, and Campylobacter peaked between April and August, suggesting a seasonal effect. The 0–3 years age group had the highest number of identified cases, accounting for 43.3% (261/603) of total detections. Positive identifications were more frequent among females (56.2%, 339/603). Pediatric emergency department recorded the highest number of detected agents 59.4% (358/603), emphasizing the burden of gastrointestinal infections among children.

The adoption of multiplex real-time PCR assays in the diagnosis of gastrointestinal infections has identified gaps and improved the rates of detection for multiple pathogens. The implementation of a syndromic testing panel can therefore provide healthcare professionals with timely and accurate information for more effective treatment and public health interventions.

C017

EPIDEMIOLOGY OF RESPIRATORY VIRUSES: INSIGHTS FROM THE MAIN HOSPITAL IN THE AZORES ARCHIPELAGO, PORTUGAL

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Respiratory viruses are among the most important pathogens affecting global health, particularly in children and elders. Coinfections complicate diagnosis and treatment, aggravate patients' clinical conditions, and increase the pressure on healthcare systems.

In this study, we aimed to analyse the prevalence and distribution of Influenza A (H3, H1N1pdm09), Influenza B, Respiratory Syncytial Virus (RSV), Human Coronaviruses (229E, NL63, HKU1, OC43, SARS-CoV-2), Rhinovirus, Adenovirus, Enterovirus, and Metapneumovirus. Additionally, we investigated the epidemiological dynamics of these pathogens, the coinfection prevalence and seasonal pattern of respiratory infections in the Azores archipelago, comparing the data obtained with that from mainland Portugal (through INSA). We analysed data from 2023 and 2024.

In 2023, a total of 9755 tests were performed for SARS-CoV-2, 6422 for Influenza A/B and RSV, and 1103 for other respiratory viruses. The peak of SARS-CoV-2 cases occurred in January (180 cases), with the lowest count in November (53). Influenza A(H1N1)pdm09 was predominant, and RSV had the highest incidence between November and January.
Among the remaining viruses, Rhinovirus was the most frequent (245 cases), peaking in December (50). The most common coinfections included Adenovirus and RSV followed by Rhinovirus and RSV.

In 2024, 4002 tests were conducted for SARS-CoV-2, 4914 for Influenza A/B and RSV, and 979 for other viruses. The peak of SARS-CoV-2 occurred in June (79 cases), with the lowest count in December (3). Influenza B was the most prevalent, with 118 cases in December. Rhinovirus remained the most frequently detected (214 cases), followed by Adenovirus (87). All respiratory agents were detected at least once.

The number of tests declined in 2024, in line with the decreasing trend of SARS-CoV-2 in the EU/EEA, and was further impacted by a fire in our hospital, which affected testing requests (Azores is still in calamity state). Comparing these results with those reported for mainland Portugal, we observed a delay in the onset of the "flu season" in the Azores. This finding may help optimise the timing of vaccination programs for high-risk age groups, in the Azores islands.

Finally, this study highlights the diversity of respiratory viruses and coinfections, providing valuable insights for public health measures and contributing to the development of more effective control and prevention strategies

CO18

ATYPICAL BACTERIAL RESPIRATORY INFECTIONS IN CHILDREN

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Introdution: Mycoplasma pneumoniae and Chlamydophila pneumoniae commonly cause mild infections of the respiratory system. M. pneumoniae infections are most common in young adults and school-aged children. The most common manifestation of M. pneumoniae infection is tracheobronchitis (chest cold). Common symptoms of a chest cold include sore throat, tiredness, fever, and slowly worsening persistent cough.

Objective: Atypical bacteria have been responsible for up to 23% of pneumonias in children. This resume aims to discuss the diagnostic criteria, management and preventive measures related to atypical respiratory infections in children.

Material and Methods: Between 2020 and 2024, approximately 8640 patients realized Multiplex Respiratory PCR test – BioFire Panel RP2.1plus – which uses a syndromic approach to accurately detect and identify the pathogens most commonly associated with viruses and bacteria of respiratory infections (for example, M. pneumoniae and C. pneumoniae), on the BioFire® FilmArray® 2.0. (bioMérieux, France), with 1502 patients with at least one pathogen detected.

Results: Sampled were mostly from male (n =106; 58.2%) in cases of M. pneumoniae and are from female (n =26; 51%) in cases of C. pneumoniae.
There were 19 samples from children bellow 1 y/o (10.4%), 83 samples of patients aged between 1-5 y/o (45.6%), 58 samples of patients aged between 6-10 y/o (32%) and 22 samples of patients aged between 11-17 y/o (12%) in cases with M. pneumoniae. In cases of C. pneumoniae there were 2 samples from children bellow 1 y/o (3.9%), 20 samples of patients aged between 1-5 y/o (39.2%), 18 samples of patients aged between 6-10 y/o (35.3%) and 11 samples of patients aged between 11-17 y/o (21.6%).
In Atypical respiratory infections with M. pneumoniae, respiratory manifestations are pneumonia, acute bronchitis; upper respiratory tract; infection; asthma exacerbation; with C.

pneumoniae are pneumonia, rhinitis, sinusitis, pharyngitis, laryngitis and acute bronchitis. Whit insidious onset; non-productive cough; low-grade fever; in children > 3-5 years old; no response to usual treatment; perihilar and bilateral pulmonary infiltrate with wheezing.

Conclusions: In conclusion, pneumonia caused by atypical pathogens is typically mild and has long-lasting symptoms. Patients frequently recover during treatment without complications.

CO19

ACUTE GASTROENTERITIS CAUSED BY SALMONELLA ENTERICA SPP. ENTERICA WITH HEMATOGENOUS DISSEMINATION: THE IMPORTANCE OF LABORATORY DIAGNOSIS

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Introduction: Acute gastroenteritis (AGE) caused by Salmonella enterica can progress to severe forms, particularly in immunocompromised patients. This case highlights the crucial role of laboratory diagnosis in complicated AGE with hematogenous dissemination.
Case Description: A 70-year-old male with a history of lung adenocarcinoma (lobectomy in 09/2023, stage IIB, PDL1-100%) and completed adjuvant chemotherapy in 12/2023 was admitted with profuse diarrhea and vomiting for three days, without fever, presenting with hypotension and severe dehydration. Laboratory findings showed AKIN III acute kidney injury, metabolic acidosis, hypokalemia, and hypomagnesemia.
Salmonella enterica spp. enterica was identified in both stool and blood cultures using MALDI-TOF MS, and antimicrobial susceptibility testing (AST) was performed. Ceftriaxone therapy was initiated. The patient developed acute confusional state and renal dysfunction, requiring intensive electrolyte correction. After 14 days of antibiotic therapy, he was discharged.

Discussion: Hematogenous dissemination of Salmonella enterica is rare and typically occurs in patients with predisposing factors such as immunosuppression and malignancies.
Laboratory evaluation was crucial in identifying the causative microorganism and obtaining AST results, allowing for targeted antibiotic therapy. It also played a key role in managing renal dysfunction and electrolyte disturbances.

Relevance: This case underscores the essential role of laboratory testing in complicated AGE, emphasizing the importance of a multidisciplinary approach in oncologic patients.

Key Takeaways: AGE caused by Salmonella enterica in immunocompromised patients can have a severe course, requiring early laboratory diagnosis and intervention to optimize prognosis.

Key words: Complicated gastroenteritis, Hematogenous dissemination, Laboratory diagnosis

CO20

EVALUATION OF IN VIVO INTERFERENCE OF DIPYRONE IN DETERMINATIONS OF URINARY BIOCHEMICAL PARAMETERS

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Introduction: Dipyrone is a widely used medication for pain and fever. After absorption, dipyrone is rapidly hydrolyzed and produces four main metabolites: 4-methylaminoantipyrine (4-MAA), 4-aminoantipyrine (4-AA), 4-acetylaminoantipyrine (4-AAA) and 4 - formylaminoantipyrine (4-FAA). Some studies indicate that dipyrone and its metabolites may interfere in vitro with the determination of serum biochemical parameters.

Objective: In this context, the aim of this study was to evaluate the in vivo interference of dipyrone in the determination urinary biochemical parameters as glucose, hemoglobin, nitrite and protein.

Methods: Urine samples from 20 healthy individuals were collected before and 1.5, 5, 7 and 13 hours after the ingestion of 1.5 g dipyrone. Using WAMA and INLAB reagent strips, the urinary parameters hemoglobin, glucose, proteins and nitrite were evaluated after the addition of known quantities of each analyte in each of the samples collected at different times after the consumption of dipyrone. A change in the result in more than one measurement unit in the majority of participants or a change in diagnostic result from positive to negative was considered significant. The urinary concentration of dipyrone metabolites at different times after the dose administration of the 1500 mg of medication was measured by high-performance liquid chromatography.

Results: The results demonstrated that there was significant interference in the semi-quantification of urinary hemoglobin by the WAMA strip up to 13 hours after ingestion of 1500 mg of dipyrone, not being time or concentration dependent.
Although not significant, some individuals presented interference in the determination of urinary protein and glucose with both strips.

Conclusion: The results indicate that urine samples should be collected before administration of dipyrone, or that administration of dipyrone should be suspended at least 13 hours before urine collection. If it is not possible to suspend the use of dipyrone, caution is suggested in interpreting the results.

CO21

EFFECT OF HYPERBARIC OXYGEN THERAPY ON THE MODULATION OF THE INFLAMMATORY RESPONSE IN AN EXPERIMENTAL SEPSIS MODEL

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Introduction: Sepsis is a critical medical condition characterized by a dysregulated systemic inflammatory response, associated with high morbidity and mortality. Hyperbaric oxygen therapy (HBOT) has been studied as an adjunctive treatment due to its anti-inflammatory and antioxidant properties.

Objective: To evaluate the impact of HBOT on clinical, haematological, immunological, and biochemical parameters in Wistar rats subjected to lipopolysaccharide (LPS)-induced sepsis.

Materials and Methods: Eighteen male Wistar rats (250 ± 20 g) were divided into three groups: control, LPS (10 mg/kg, intraperitoneally), and LPS + HBOT. The LPS + HBOT group underwent a single HBOT session (2.5 ATA for 90 minutes) three hours after LPS administration.
Animal behaviour, clinical signs, haematological parameters (leucogram and erythrocyte sedimentation rate), inflammatory cytokines (TNF-α, IL-6, IL-1, IL-10), and markers of liver (AST, ALT) and renal function (creatinine, urea) were assessed. Statistical analysis was performed using parametric and non-parametric tests for group comparisons, with $p < 0.05$ considered statistically significant.

Results: Rats in the LPS + HBOT group maintained normal behaviour, whereas the LPS group exhibited lethargy and yellowish discolouration. There was a significant reduction in leucocytosis (LPS: 15 ± 2 vs. LPS + HBOT: 11 ± 1.5; $p < 0.05$) and erythrocyte sedimentation rate (LPS: 25 ± 5 vs. LPS + HBOT: 15 ± 4 mm/h; $p < 0.05$). Levels of TNF-α (LPS: 210 ± 30 vs. LPS + HBOT: 130 ± 20; $p < 0.05$), IL-6 (LPS: 180 ± 25 vs. LPS + HBOT: 115 ± 18; $p < 0.05$), and IL-1 (LPS: 220 ± 28 vs. LPS + HBOT: 130 ± 20; $p < 0.05$) were significantly decreased, while IL-10 levels were significantly increased (LPS: 90 ± 10 vs. LPS + HBOT: 140 ± 15; $p < 0.05$) in the HBOT-treated group. Liver enzymes (AST: LPS: 120 ± 20 vs. LPS + HBOT: 80 ± 15; ALT: LPS: 115 ± 18 vs. LPS + HBOT: 75 ± 12; $p < 0.05$) and renal markers (creatinine: LPS: 1.5 ± 0.2 vs. LPS + HBOT: 1.1 ± 0.1; urea: LPS: 45 ± 7 vs. LPS + HBOT: 30 ± 5; $p < 0.05$) also showed significant improvements in the HBOT group.

Conclusion: HBOT demonstrated beneficial effects in modulating the inflammatory response and improving clinical and laboratory parameters in Wistar rats with LPS-induced sepsis. These results suggest that HBOT may represent a promising therapeutic approach in the management of sepsis, warranting further research to confirm its clinical potential.

CO22

IMMUNOLOGICAL ADAPTATIONS DURING PREGNANCY: INSIGHTS INTO LYMPHOID DYNAMICS AND THE NEED FOR REFERENCE RANGES

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Introduction: During pregnancy, the maternal immune system undergoes complex changes, balancing tolerance and defence to ensure a successful outcome. Innate and adaptive immune responses are modulated throughout the 3 trimesters of gestation, but the specificities of these changes are not fully understood.
A clear perception of the trimester-specific immunological profiles and the establishment of reference ranges is crucial to improve maternal and prenatal care, allowing early detection of deviations linked to poorer outcomes and pregnancy-related complications.

Methods: Peripheral blood samples from 50 healthy pregnant women (PW) in the 1st (T1), 2nd (T2), and 3rd trimesters (T3) were analysed, with 30 healthy non-pregnant women (NPW) of similar age recruited as controls. A detailed characterization of immune cell subsets and activation markers was performed by flow cytometry.

Results: Several changes were observed in erythrogram and leucogram components throughout pregnancy, although mean values remained within normal ranges. Namely, neutrophil and monocyte counts increased progressively from T1, while lymphocytes remained stable but lower than in NPW. Within lymphocytes, transitional and anergic naïve B cells declined progressively, especially in T3, while activated naïve and switched memory B cells increased, suggesting heightened activation. Significant increases in activated CD4 and CD8 T cell subsets were observed, especially prominent in T2 and T3, both within trimesters and relative to NPW, underscoring their critical role in immune adaptation during these stages. Stimulation studies confirmed higher baseline activation levels in B and T cells during pregnancy, particularly at T3. Furthermore, PW exhibited increased expression of activation markers after stimulation in CD4 and CD8 T cells, compared to NPW, with the most pronounced differences in T3.

Conclusions: Our findings reveal previously unrecognized immunological variations during pregnancy, highlighting distinct immune profiles compared to non-pregnant women.
Pregnancy reportedly influences the lymphoid compartment and specific subsets, despite shifts in parent populations may fall within normal ranges.
We emphasize the need for adjusted reference values for specific subsets along the trimesters of pregnancy to enhance their clinical applicability.

CO23

FLUCONAZOLE THERAPEUTIC MONITORING: REPORT OF A LIFE-SAVING CASE IN A CRYPTOCOCCUS MENINGOENCEPHALITIS CONDITION

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Introduction: Cryptococcus is a globally distributed, opportunistic, yeast-like fungus that primarily infects immunocompromised individuals. When it reaches the central nervous system (CNS) becomes life-threatening potentially causing meningoencephalitis.
First-line induction therapy combines amphotericin B and flucytosine. If contraindicated, fluconazole is used as monotherapy, or with the first line drugs.
Due to the high interindividual variability, therapeutic drug monitoring (TDM) is recommended for all antifungals. The clinical case reports a successful fluconazole dosing for a Cryptococcal meningoencephalitis after TDM.

Case Description: 62-year-old woman referred to emergency room, after a screening positive test for Human Immunodeficiency Virus (HIV) and seizures. The laboratory tests confirmed HIV-1 and the presence of Cryptococcus sp. in cerebrospinal fluid (CSF).
She was hospitalized and started therapy with amphotericin B (200mg id) and flucytosine (1g 6/6h).

During hospitalization, she developed a maculopapular rash, suspected to be an allergic reaction to amphotericin B. Treatment was switched to Fluconazole (1200mg id).
The clinical condition of meningoencephalitis worsened which could be related to toxicity since the symptoms overlap. Fluconazole was dosed after 8 days of treatment. The results revealed a toxic blood concentration of 90.1mg/L (therapeutic range: 20-50mg/L).
The dose was reduced to 800 mg id and the serum levels dropped to 55.6mg/L (day 22) and to 47.2mg/L (day 35). The patient was discharged 53 days later with an improved condition.

Discussion: This case highlights the importance of TDM in optimizing fluconazole dosing for the treatment of Cryptococcal meningoencephalitis in an immunocompromised patient for the entire duration of treatment. Standardized dosing may not be suitable for all patients, especially in prolonged therapy. In this particular case, serum dosing proved to be life-saving, as the symptoms caused by fluconazole therapy toxicity overlapped with the preexisting clinical condition. If TDM had not been performed, it would be easy to to attribute the entire clinical picture to meningoencephalitis. This reinforces the importance of individualized pharmacokinetic monitoring for balancing efficacy and safety.

CO24

URINARY SEDIMENT ANALYSIS AS A KEY DIAGNOSTIC TOOL: CASE REPORT OF LUPUS NEPHRITIS WITH LEUKO-ERYTHROCYTIC CASTS AND DYSMORPHIC ERYTHROCYTES

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Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect various organs, including the kidneys. Urinalysis is essential in SLE follow-up, as lupus nephritis (LN) may occur before the onset of overt renal symptoms. Early diagnosis is crucial to prevent irreversible damage. Urinalysis must include the detection of proteinuria, haematuria and urinary sediment analysis, in which pathologic casts, dysmorphic erythrocytes, lipid droplets and Renal Tubular Epithelial Cells (RTEC) constitute important warning signs of renal involvement. These findings justify the need of renal biopsy for accurate diagnosis and treatment guidance.

Case Description: A 32-year-old woman was diagnosed with SLE in 2006 after presenting with hemolytic anemia and nephritic syndrome. A renal biopsy at the time revealed a class IV LN, which was treated and remained in remission.
In October 2024, the patient presented with rosacea and started vibramycin. Days later, she developed inflammatory arthralgia in her hands, feet and ankles. Laboratory tests revealed anti-dsDNA antibodies 54.0 IU/mL, anti-C1q antibodies 80.3 U, serum creatinine 0.86 mg/dL, urea 28 mg/dL, cystatin C 1.13 mg/L and Larsson eGFR 66 mL/min. Urinalysis by dipstick showed proteinuria (300 mg/µL), haemoglobinuria (0.15 mg/dL) and leukocyturia (75 leu/µL).
Urinary sediment examination by phase-contrast microscopy revealed mild leuco-erythrocyturia with dysmorphic erythrocytes, hyaline-granular and leukocyte-erythrocytic casts, lipid droplets and RTEC. Subsequent renal biopsy disclosed a focal class III LN, guiding treatment with anti-inflammatory and immunosuppressive therapy.
Discussion: Early detection of LN is essential to optimize treatment and prevent progression to renal failure in SLE patients.
Regular monitoring of serological markers combined with GFR, proteinuria, haemoglobinuria and urinary sediment evaluation helps detect renal complications
allowing early intervention and thus reducing progression to severe and irreversible disease.

Dysmorphic erythrocytes, leuko-erythrocytic casts, lipid droplets and RTEC in urinary sediment are sensitive, valuable, non-invasive and cost-effective markers of renal injury, justifying renal biopsy for confirmatory diagnostic and histopathological classification.
This case highlights the importance of systematically incorporating urinalysis into SLE follow-up protocols, promoting better clinical outcomes.

CO25

CHATGPT - TAMING THE WILD HORSE ON LABORATORY MEDICINE

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Introduction: The EU AI Act (1) is the world's first regulatory framework for Artificial Intelligence (AI), emphasizing that "AI systems should be overseen by people." The EFLM AI Working Group evaluated ChatGPT on interpreting laboratory results and identified limitations due to "being not specifically trained on medical data or laboratory data in particular" (2). Furthermore, analytical methods with measurement uncertainty (IM) below the intraindividual biological variation (CVi) of a biological marker are deemed safe for clinical decision-making (3).

Objective: This study seeks to determine if ChatGPT can be reliably used by non-laboratory professionals for decision-making concerning a potential new potassium (K) dosing method in a clinical laboratory setting.

Materials and Methods: We utilized ChatGPT (free Version 4) (GPT4) for consultations by simulated middle management hospital staff, posing the initial question: "Plot a chart comparing the uncertainty of measurement (IM) of potassium dosing methods with the known intraindividual biological variation (CVi) in humans over the past 30 years." A similar question was asked after a senior laboratory medicine specialist, with over 15 years of experience in biological variation, provided additional technical guidance, asking to include data from the AEFA External Quality Assessment Programs, the SEQC Biological Variation Database (BVD), and the new EFLM BVD, all with their respective confidence intervals.

Results: Significant differences were observed in the graphics generated by GPT4. Figure 1, produced by the simulated hospital middle management non-laboratory professional, showed a steadily decreasing IM for potassium dosing methods, stops at around 2% IM in 2022 (last data for GPT4), with an almost constant CVi of approximately 7%. This suggests a simple management decision to adopt any method with 7% IM or lower for K. In contrast, Figure 2, generated by the senior laboratory specialist, revealed two major shifts: from a median IM of 4% and a maximum CVi of 5% in 2000, to a median IM of 1.5% and a maximum CVi of 4,5% (from 2015) in 2010.
This indicates that the state of the art for K is a stabilised median IM of 1.5% since 2010, with a recommended upper limit of 4,5% IM for safe clinical decisions.

Conclusion: Despite the efforts exemplified by the EU AI Act, trained laboratory medicine specialists are essential to effectively utilize GPT4 for laboratory management decisions, ensuring patient safety.

CO26

OPTIMIZING HEPATOCELLULAR CARCINOMA DIAGNOSIS: IMPLEMENTATION OF THE GAAD ALGORITHM

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The GAAD is an in vitro multivariate index assay that provides a semiquantitative result to aid in the early detection of Hepatocellular Carcinoma (HCC). It integrates an algorithm combining α-fetoprotein (AFP) and PIVKA-II (Protein Induced by Vitamin K Absence or Antagonist-II) assays in human serum/plasma, along with the patient's gender and age. This score is intended for adults with manifestations of chronic liver disease, those with increased risk of developing HCC and for the detection of both early- and all-stage HCC.
GAAD results should be interpreted alongside clinical findings and other diagnostic tools, such as liver image study. Our hospital center was the first within the National Health Service to implement the GAAD algorithm.

A total of 60 patients (males 66.7% and females 33.3%) followed up in the Hepatology Clinic were enrolled in this study; 80% (48/60) had liver cirrhosis (LC) and 20% (12/60) had HCC. The mean age was 64.5 years for the LC group and 63.3 years for the HCC group.
Serum levels of Elecsys PIVKA-II (ng/mL) and Elecsys AFP (ng/mL) were measured using the Cobas® e 801 analyzer (Roche). The predefined cut-off values were 9.68 ng/mL for the AFP assay and 131 ng/mL for the PIVKA-II assay. Additionally, the Elecsys GAAD algorithm was applied, with a cut-off value set at 2.57. The results were analysed using IBM® SPSS® Software. A comparative analysis was conducted to assess differences between the AFP assay, the PIVKA-II assay and the GAAD algorithm.

These results constitute the first measurement for each patient. In the LC group, the AFP assay yielded 8.3% of abnormal results, while in the HCC group was 50%. The PIVKA-II assay showed 35.4% abnormal results in the LC group and 41.7% in the HCC group. When applying the GAAD algorithm, abnormal results were 41.7% within LC and 83.3% within HCC.Abnormal AFP levels in LC patients highlight its limited utility in suggesting malignant transformation. The PIVKA-II assay demonstrated greater sensitivity in predicting HCC compared to AFP. However, the GAAD algorithm outperformed both AFP and PIVKA-II in predictive accuracy.
While AFP alone failed to detect half of the HCC cases, PIVKA-II achieved a higher detection rate but remained suboptimal. Among the three methods, the GAAD algorithm exhibited the highest detection rate, making it the most promising tool for HCC prediction. Further results will enhance our understanding of the GAAD algorithm and its role in HCC diagnosis.

CO27

ALLERGIC SENSITIZATION PROFILE IN PATIENTS WITH ANAPHYLAXIS CLINIC AFTER HYMENOPTERA STING, IN A POPULATION OF A REGION OF PORTUGAL

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Introduction: The most frequently implicated venoms in hymenoptera allergy are: bee (Apis mellifera, AM), wasp (Vespula spp., VV) and European paper wasp (Polistes dominula, PD).

The respective major molecular allergens, Api m1, Ves v5 and Pol d5, are important in distinguishing between primary sensitization or double sensitization.

Objectives: To evaluate the allergic sensitization profile to the most frequent hymenoptera and identify which are the most prevalent major molecular allergens, as well as the usefulness of their measurement, in the population of a region of Portugal.

Methods: Search the database of the Clinidata® software program, between 2019 and 2024, for the results of specific IgE (sIgE) measurements to the total extract of the 3 main hymenoptera, and sIgEs to molecular allergens. sIgE was measured by immunoenzymatic methods.

Results: Of the 126 patients who underwent sIgE measurement to the total extract of the 3 main hymenoptera, values >0.35kU/L (reference value <0.35kU/L) had the following distribution: 98 (77.8%) to VV, 83 (65.9%) to PD and 81 (64.3%) to AM.
The allergen with the highest measurement in each patient was VV in 43 patients, AM in 39 patients, PD in 27 patients and equal for VV and PD in one patient.
In 16 patients the measurements were <0.35 kU/L.
Of the 108 patients who underwent sIgE measurement to molecular allergens, values >0.35 kU/L had the following distribution: 64 (59.3%) rVes v5, 59 (54.6%) rPol d5 and 27 (25%) rApi m1.
The allergen with the highest measurement in each patient was rVes v5 in 39 patients, rPol d5 in 22 patients, rApi m1 in 21 patients and equal value for rVes v5 and rPol d5 in one patient. In 25 patients the measurements were <0.35 kU/L.
There was found agreement with the molecular allergens and the total extract in 61 patients (56.5%) and in 22 (20.4%) there was disagreement, of which in 11 of these it was between bees and vespids. In 16 patients, sIgE to total extract was positive, but negative to molecular allergens. In 9 patients, sIgE to total and molecular extract were negative. No patient with negative sIgE to total extract had positive sIgE to molecular allergen.

Discussion: In this population, the majority of patients are sensitized to vespids. This study showed that between the sIgE measurement to total and molecular extracts there was 20% disagreement, demonstrating the importance of the sIgE measurement of the molecular extracts in assisting diagnosis and therapeutic guidance

CO28

QUANTIFICATION OF THIOPURINE METABOLITES IN ERYTHROCYTES: A KEY FOR OPTIMIZING AZATHIOPRINE THERAPY AND CLINICAL OUTCOMES

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Introduction: The prodrug azathioprine (AZA) is an immunosuppressive drug. After an oral dose, AZA degrades to mercaptopurine, which, upon activation, originates the thioguanine nucleotides (TGN), acting as purine antagonist.

TGN inhibits DNA, RNA and protein synthesis, inducing cytotoxicity and immunosuppression. Simultaneously 6-methylmercaptopurine (6MMP) is also formed, which may be responsible for some toxic effects such as hepatotoxicity.
AZA is considered a critical-dose drug because it is used to treat critical disease states and demonstrates wide interpatient variability in drug metabolism which may influence patient responsiveness to therapy and patient susceptibility to drug-induced cytotoxicity (1,2).

Objective: Evaluate the results of AZA metabolites after implementing an LC-MS/MS method for therapeutic monitoring.

Materials and Methods: An anonymized parametric search was carried out on the samples in which AZA metabolites were determined. 6MMP and 6TG (representing the TGN) were quantified, considering the references: 235-450 pmol/8x108 R.B.C. for 6TG, <5700 pmol/8x108 R.B.C. for 6MMP and 5-25 for 6MMP/6TG ratio.
Elevated 6MMP levels and increased ratio are associated with higher risk for hepatotoxicity and relapse. High levels of 6-TG are associated with drug-induced leucopenia (3).

Results: The metabolites of AZA were evaluated in ten patients (7 women; age range 17-62 years) with autoimmune hepatitis. Regarding 6MMP/6TG ratio, seven patients presented reduced and three had normal ratio. For 6TG, only two patients presented an optimal value, the others were reduced. None had toxic concentration of 6MMP.
Leukopenia was present in one patient, thrombocytopenia in two, elevated IgG in four and increased transaminase activity also in four.
Three patients had obesity, with one experienced a few relapse episodes in the last year, possibly indicating low compliance, and one other, cutaneous lesion. None reported gastrointestinal disturbances.

Conclusion: Monitoring AZA and its metabolites is crucial to understand the clinical evolution of patients. The results indicated that some patients are not clinically controlled, presenting increased transaminase activity and low 6TG, which can be due to inappropriate dose of AZA, no compliance or the presence of genetic polymorphisms.
Genotype assessment can provide valuable insights but due to the interpatient variability, it is crucial to monitories AZA metabolites, enhancing safety and efficacy.

CO29

COMMON VARIABLE IMMUNODEFICIENCY OR STAT3 GOF SYNDROME? RETHINKING THE DIAGNOSIS OF INBORN ERRORS OF IMMUNITY THROUGH GENETIC TESTING

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Hypogammaglobulinemia has a broad differential diagnosis, making it crucial to distinguish primary from secondary causes. Common variable immunodeficiency (CVID) is the most common symptomatic inborn error of immunity (IEI) in adults and is a diagnosis of exclusion.
STAT3 gain-of-function (GOF) syndrome and CVID share overlapping features, including hypogammaglobulinemia, abnormal vaccine responses, and recurrent infections. However, STAT3 GOF is strongly associated with autoimmunity, lymphoproliferation and failure to thrive.

It can also present with cortical and subcortical enhancing brain lesions—an uncommon feature in CVID. A definitive diagnosis requires genetic testing.

A 41-year-old female, diagnosed with CVID at 33, had a history of recurrent respiratory infections since childhood with growth delay. Laboratory findings showed hypogammaglobulinemia, lymphopenia with reduced CD4+ T, NK, B, and switched B cells, and increased CD21 low lymphocytes. She exhibited hepatosplenomegaly, lymphadenopathy, autoimmune hemolytic anemia, alopecia areata, and interstitial lung disease classified as CVID associated granulomatous lymphocytic interstitial lung disease (GLILD).
However, several atypical findings emerged: absence of significant infections in adulthood, severe corticosteroid-refractory cytopenia, IgG levels >400 mg/dL at diagnosis and nonspecific granulomatous cerebral lesions responsive to corticosteroids. These were presumed to be extrapulmonary GLILD due to the CVID diagnosis and inability to biopsy, though cerebral involvement is highly atypical. A CVID NGS panel was performed, yielding negative results; however, an IEI panel identified a STAT3 GOF mutation, leading to a revised diagnosis.

An accurate diagnosis is critical, as STAT3 GOF syndrome requires tailored immunosuppressive therapy alongside immunoglobulin replacement. The prognosis also differs, with a higher risk of autoimmunity and complications demanding closer monitoring.
Misdiagnosing STAT3 GOF as CVID leads to suboptimal care. As our understanding of IEI genetics evolves, reassessing past diagnoses is essential to ensure optimal treatment.

CO30

ARE CURRENT ALBUMINURIA CUTOFFS TELLING THE FULL STORY? - A SYSTEMATIC REVIEW OF THE ASSOCIATION BETWEEN ALBUMINURIA AND CARDIOVASCULAR RISK

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Introduction: Albuminuria is widely used to identify diabetic individuals at risk for nephropathy and to assess cardiovascular risk, particularly in hypertensive patients.
Recent evidence highlights its importance as a key marker of cardiovascular risk, extending beyond hypertensive and diabetic populations to the general population.

Aims: To investigate the association between albuminuria and cardiovascular outcomes and to identify cutoff points that best predict cardiovascular events and markers of morbidity in populations with at least one cardiovascular risk factor.

Methods: A comprehensive literature search of PubMed, Scopus, and ScienceDirect was conducted up to October 1, 2024, for articles published between January 2014 and October 2024, following PRISMA guidelines.
The included studies explored the association between albuminuria and cardiovascular morbidity and mortality, identifying specific albuminuria cutoffs that best predict an increased cardiovascular risk or markers of morbidity. The risk of bias was evaluated using the Joanna Briggs Institute Critical Appraisal Tools for Cross-Sectional Studies and for Cohort Studies.

Results: Of the 1 432 studies initially selected, 9 met the eligibility criteria and were included in this systematic review, encompassing a total of 120 149 participants. This systematic review primarily focuses on individuals with Diabetes Mellitus and Hypertension, as the studies meeting

all inclusion criteria primarily address these conditions. Albuminuria was associated with various cardiovascular outcomes, including cardiovascular mortality, cardiovascular diseases, left ventricular hypertrophy and dysfunction, and acute myocardial infarction. The included studies proposed albuminuria cutoff points ranging from 0.7 mg/mmol to 2.26 mg/mmol for diabetic adults, and from 0.59 mg/mmol to 2.26 mg/mmol for hypertensive adults. A meta-analysis was not conducted due to the heterogeneity of the studies.

Conclusions: This review confirms the association between albuminuria and cardiovascular outcomes in both diabetic and hypertensive adults, even at levels below those used in current categorization frameworks.

CO31

CAUSES OF SAMPLE REJECTION IN A CLINICAL ANALYSIS LABORATORY OF A UNIVERSITY HOSPITAL

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Introduction: The pre-analytical phase of clinical laboratory is the most susceptible stage to failures due to lower automation and greater number of professionals involved, resulting in 40% to 70% of laboratory errors.
To ensure, monitor, and guarantee quality in this phase, the use of Quality Indicators (QI) is recommended. One of the most commonly used QIs is the sample recollection rate that results in delayed diagnosis or treatment. Objective: To analyze the frequency and causes of sample rejection that lead to sample recollection in a clinical analysis laboratory of an academic hospital.
Methods: The samples indicated for recollection were considered between June 2022 and June 2024. Sample nonconformities were classified into 17 QI from the pre-analytical phase (registration error, identification error, coagulated sample, contaminated sample, hemolyzed sample, accidental sample, insufficient volume, incorrect sample, loss of sample stability, inadequate transportation and/or storage, presence of fibrin or lipemia, need for result confirmation, expired tube, collection performed outside the time indicated for the requested test, patient did not follow laboratory recommendations, lack of medical order/mandatory documentation, test not carried out on duty, others and unspecified).
The QI were calculated considering the number of occurrences in relation to the number of tests performed in the period, expressed in percentage, and by Sigma metric. Each of the QIs was also categorized according to the analytical sector and area of origin of the sample.

Results: During the period analyzed, 1,209,489 tests, and 910 recollections were requested (0.07%, Sigma 4.7).
The main causes for sample recollection were: clotted sample (312/910, 34.0%), insufficient volume (159/910, 17.2%), and result confirmation (87/910, 9.8%). The sectors that most requested sample recollection were: Hematology (417/910, 45.8%), Biochemistry (222/910, 24.3%) and Screening (81/910, 8.9%).
The areas with the highest frequency of sample recollection were Outpatient Clinic (134/910, 14.7%), Neonatal ICU (121/910, 13.3%) and Adult Emergency (114/910, 12.5%).

Conclusions: The causes and frequency were similar to those described in the literature, and presented acceptable rates, indicating quality.

However, the causes of sample recollection appear to be due to internal laboratory failures, indicating opportunities for process improvement.

CO32

ORGANOPHOSPHORUS INSECTICIDE SELF-POISONING – AN UNEXPECTED EVENT

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Introduction: Organophosphorus (OP) toxicology evolved from the typical acetylcholinesterase (AChE) inhibition by OP insecticides and chemical warfare agents to various primary and secondary mechanisms for several OP herbicides, fungicides, pharmaceuticals, and industrial chemicals. The authors present a clinical case of self-poisoning with OP insecticide (chlorpyrifos).

Clinical case: A 74 year-old male that lived in a rural area voluntarily ingested an OP insecticide in a suicide attempt. He was admitted in the hospital severely ill. Clinically, he developed a typical presentation of OP intoxication, encompassing these 3 stages, in order of appearance: cholinergic crisis; intermediate syndrome; and OP-induced delayed neuropathy. His peripheral blood samples and urine samples, tested using Liquid Chromatography–Mass Spectrometry (LC-MS) and Gas Chromatography–Mass Spectrometry (GC-MS), were found to be positive for chlorpyrifos.

Discussion: Chlorpyrifos is an OP insecticide, which is a monoacylglycerol lipase (MAGL) inhibitor leading to the cannabinoid syndrome under conditions in which 2-arachidonoylglycerol (2-AG) accumulates on MAGL inhibition. It can also inhibit endocannabinoid metabolizing enzymes in animal models at levels that do not significantly alter AChE in central nervous system.
OP insecticide self-poisoning is an important clinical problem in many countries.
Its prevention should involve regulation to remove the most hazardous pesticides from agricultural practice, and improved use and storage of pesticides. Its treatment requires adequate medical management.
Although chlorpyrifos is banned in the European Union since 2020, it is still possible to find this insecticide. The authors emphasize the need to comply with current regulations concerning phytosanitary products.

CO33

HYMENOPTERA VENOM ALLERGY AND BASELINE SERUM TRYPTASE

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Introduction: Serum tryptase is a marker of mast cell degranulation; elevated levels help confirm mast cell activation during a severe allergic reaction, such as anaphylaxis, and in the diagnosis of systemic mastocytosis.

Hymenoptera venoms contain potential allergens and can cause exuberant local and/or systemic allergic reactions in sensitized individuals.
Patients with elevated baseline serum tryptase (BST) levels may experience more severe episodes of allergic reactions after a hymenoptera sting.

Objectives: To evaluate BST levels in a population of patients with hymenoptera venom allergy followed at the Allergy and Clinical Immunology Clinic of an institution. To identify whether elevated BST is correlated with allergic sensitization to any specific hymenoptera venom.
Materials and methods: The Clinidata® database was searched for the results of specific IgE (sIgE) assays for hymenoptera venoms (Apis mellifera [AM], Vespula spp. [VV], and Polistes dominula spp. [PD]) requested at the Allergy and Clinical Immunology Clinic for patients with a history of anaphylaxis after a hymenopteran sting. In this population, BST values were measured in the same sample as the serum sIgE determination for hymenoptera.
It was statistically evaluated whether BST is correlated with: sIgE assays for the 3 hymenoptera tested (Pearson's Correlation Coefficient test, PSPP 2.0.1); and with the hymenoptera with the highest sIgE assay per patient (ANOVA test, PSPP 2.0.1).

Results: The analytical parameters of 126 patients were evaluated. The mean BST value was 5.20±4.44ng/mL (minimum 1.4ng/mL and maximum 46.2ng/mL). A BST>11.4ng/mL was identified in 4 patients, 2 with predominant sensitization to bee and 2 to wasp. A very weak negative statistical correlation was found between the sIgE levels of the 3 hymenoptera tested and BST assays: Pearson's correlation for AM of -0.056 (p=0.535), for VV of -0.001 (p=0.990), and for PD of -0.083 (p=0.353). When comparing the hymenoptera with the highest sIgE assay per patient and BST, no statistical relationship was found (p=0.730).

Conclusion: No statistical correlation was found between BST levels and sensitization to the 3 hymenoptera studied, nor between BST levels and sIgE assays. The results obtained suggest that BST levels are dependent on individual factors of each patient and are not dependent on sensitization to a specific hymenoptera.

CO34

IGG SUBCLASSES - A POSSIBLE CLUE FOR GAMMOPATHY DIAGNOSIS

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Introduction: Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic clonal plasma cell disorder defined by a serum monoclonal protein of <3 g/dL, clonal bone marrow plasma cells of <10% and absence of end-organ damage or amyloidosis. Though treatment is not usually necessary, close follow-up guided by risk stratification is advised due to increased risk of progression to multiple myeloma.

Case report: A 66 year-old male patient with a history of COPD was evaluated at a Pneumology consultation after an emergency admission due to worsening dyspnoea and respiratory insufficiency.
A serum analytical panel was ordered, revealing an Immunoglobulin G (IgG) of 1170 mg/dL, while IgG subclasses (IgG-Sc) sum was of 1758 mg/dL, representing a -50% difference when compared to the total IgG level. There was also an increased IgG3 of 680 mg/dl. Our first step

was to repeat the total IgG and the subclasses assay, but we obtained similar results, even with sample predilution to minimize a possible prozone effect. We then added and measured light chains' levels, which revealed a kappa chain of 573 mg/dL, a lambda chain of 53.7 mg/dL and an elevated kappa/lambda ratio of 10.67. This raised suspicion of a monoclonal gammopathy, so we performed a serum electrophoresis - that showed a monoclonal peak of 1.73 g/dL on the gamma region – and a serum immunofixation, which identified the monoclonal protein as IgG-kappa. We then informed the clinician of our findings and the patient was referred to Hematology.

Discussion: A quarter of COPD patients have a defective humoral immunity and present with low IgG levels, leading to increased risk of infection, COPD exacerbation, hospitalization and higher morbimortality. As such, levels of immunoglobulins and IgG subclasses are part of the follow-up panel of such patients. IgG is the most frequently involved immunoglobulin in MGUS. As to IgG-Sc, patients can exhibit either normal or abnormal levels. The most frequently involved subclass is IgG1, while IgG3 and IgG4 are most often decreased. MGUS is not often diagnosed based on IgG-Sc quantification. This case is interesting because an increased level of IgG3 and different values of IgG and IgG-Sc sum raised suspicion of a potential confounding factor by the clinical pathologists, which led to the diagnosis of an unknown MGUS in this patient, allowing for adequate follow-up and monitoring.

CO35

LIPOPROTEIN(A) REFERENCE STANDARD CALIBRATION: DOES IT REALLY MATTER?

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Introduction: Lipoprotein(a)[Lp(a)] consists in a LDL particle attached to an apolipoprotein(a) and it is considered a genetic cardiovascular (CV) risk marker.
Patients classified with a certain CV risk according to traditional factors may benefit from stronger prevention strategies if Lp(a) levels are high.
European Society of Cardiology (ESC) guideline recommends isoform-insensitive methods, as Lp(a) has isoforms with different molecular mass.
However, ESC provides cut-off values for risk categorization in both molar and mass units in 6 categories.

Reference methods (Randox and Binding Site) provide results in molar units (nmol/L) as they are isoform-insensitive and their calibrator is traceable to the WHO/IFCC reference material SRM-2B. Furthermore, Randox method is certified by Northwest Research Lipid Laboratory (University of Washington).
In this work, we aim to compare our current method (Abbott – sensitive to Lp(a) isoform, with results in mg/dL) to reference methods, with focus on clinical relevance.

Methods: A prospective and unicentric study was done based on the simultaneous measurements of Lp(a) levels by three immunoturbidimetric methods in serum samples of healthy volunteers. Data was retrieved and analysed using R by Passing-Bablok regression, Bland-Altman plot and relative frequencies.

Results: We obtained a n of 58 (29 to 66 years old). Our data shows similar results between Randox and Binding Site assays (slope 1,11; Pearson's R= 0.990; mean bias 2.08 nmol/L).

Only one sample out of 53 would be classified in a different CV risk category. Randox and Abbott assays, although using different units, showed a very high correlation (slope 0,52; Pearson's R= 0.999). However, Abbott assay classified 19 patients (35%) in a higher risk category when compared to results obtained by Randox.

Conclusions: These results show that reference methods are similar, with minimal clinical difference. Unexpectedly, Abbott assay showed a strong correlation with Randox, although systematically overestimating CV risk.
This may be explained by the use of different calibrators.In the context of high demand for sample testing, Abbott assay could effectively be used as it is cheaper, has good sensitivity and high negative predictive value. When Lp(a) levels are high, they should be confirmed by a reference method in order to obtain a more accurate CV risk categorization.

CO36

ASSESSMENT OF GOOD PRACTICES FOR QUALITY ASSURANCE IN PORTUGUESE-SPEAKING COUNTRIES LABORATORIES

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Introduction: Laboratories (labs) play a fundamental role in screening, diagnosis, prognosis, and treatment of diseases. For a laboratory result to be useful, it must have guaranteed quality.
In this context, there is no informative data on the good practices adopted by labs in Portuguese-speaking countries (PLP). This information is essential for formulating policies and educational strategies intended for this target audience.

Objective: To identify and assess adherence to good laboratory practices by clinical analysis labs in PLP.

Methods: A digital questionnaire consisting of 47 questions on good laboratory practices and quality management was sent to participants in the National Program for External Quality Assessment of Portugal and other labs involved in the Laboratory Quality Improvement Project for PLP - ProMeQuaLab, except from Brazil. Data were collected anonymously between July 7 and September 30, 2024, and statistically analyzed.

Results: 59 labs (ambulatory and hospital) participated in the study, but 5 institutions did not consent to the disclosure of their data, even if anonymously. Of the 54 included labs, most were from Portugal (39; 72%), followed by Cape Verde (9; 16%), Guinea-Bissau (4; 7%), São Tomé and Príncipe (1; 2%), and 1 lab did not specify its country of origin. 57% of the labs have an implemented management system, and half of them are certified. Most labs belong to public services (63%), have a professional responsible for the management system (85%), conduct an annual training plan (85%), use quality indicators for the pre-analytical (87%) and post-analytical (83%) phases, and perform internal (70%) and external (89%) quality control.
Opportunities for improvement were identified, as only 59% of labs record the causes of rejection of control sample results, 65% develop a competency matrix, 66% construct control charts, and 72% use quality specifications to assess analytical performance.

Conclusion: Portuguese labs contributed the most to these results. Good laboratory practices are implemented, but there are opportunities for improvement.
Conducting training and involving more labs from PLP will contribute to the implementation and harmonization of good laboratory practices, which can contribute to ensuring the quality of results and patient safety.

CO37

ALPHA-1-ANTITRYPSIN DEFICIENCY: REACHING DIAGNOSIS USING PROTEIN ELECTROPHORESIS AS A SCREENING TEST

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Alpha 1 antitrypsin (AAT) deficiency is a hereditary disorder that is characterized by a low serum level of AAT. The key function of AAT is the regulation of the proteolytic effects of neutrophils elastase in the lungs. It's deficiency resulting in an increased risk of developing chronic obstructive pulmonary disease and emphysema, especially in smokers.
AAT deficiency is a rare disease that is significantly underdiagnosed (90% of patients are only diagnosed several years after the onset of symptoms). The diagnostic delay typically exceeds 5 years, resulting in an average age at diagnosis of about 45 years.
Evaluate the effectiveness of protein electrophoresis as a screening method for detecting AAT deficiency.

We carried out a retrospective review of our diagnostic results from January to December 2024. We studied 22 patients with a median age 53.3 years (24-85) with protein electrophoresis by capillary electrophoresis, AAT concentrations and alpha 1 antitrypsin genotypic characterization (Amplification of genomic DNA by PCR - Polymerase chain reaction, Sanger sequencing and specific primers for research into SERPINA1 gene variants).

Group 1: 10 patients, median age 40.6 years old (25-66), had average alpha 1 fraction value decreased (2.45 (2.9-4.9%) and 1.92 (1.9-4.3g/L)) and the screening for AAT deficiency was performed using protein electrophoresis.
This value was corroborated by the AAT assay, showing a mean value 73 (78-200mg/dL) and with the detection of 8 heterozygous variants (5S and 3Z) in the SERPINA 1 gene (80% detection). In 2 patients the results were not conclusive.

Group 2: 12 patients, median age 58 years old (42-85), were investigated for AAT deficiency by clinical suspicion. In 7 patients the diagnosis was confirmed by detecting variants (6S and 1Z) of the SERPINA 1 gene (58.3% detection). In protein electrophoresis the alpha 1 value was 2.83 (2.9-4.9%) average. Four patients were negative with normal values of alpha 1 fraction (without positive C-reactive protein) and 1 is awaiting confirmation.

The results showed that protein electrophoresis with low levels of alfa 1 fraction is a sensitive technique for suspicion of AAT deficiency (The C-reactive protein value should always be negative).

The laboratory should advise the clinician to carry out the AAT study when this changes in protein electrophoresis are observed. This may help to diagnose AAT deficiency earlier with many clinical advantages.

CO38

IS DNA GENOTYPING THE GOLD STANDARD METHODOLOGY FOR DIAGNOSIS OF HYDATIDIFORM MOLES?

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Introduction: Hydatidiform moles (HMs) are abnormal placentas characterized by trophoblast overgrowth and excessive paternal gene expression due to aberrant fertilization and gametogenesis. Partial and complete HMs are distinguishable from each other based on clinical, histologic and genetic characteristics.
Nevertheless, improvements in ultrasound technology and hCG measurement methods have led to overlapping phenotypes.
Given the inherent limitations of morphologic assessment in distinguishing molar pregnancies from other subtypes of GTD and non-molar gestations, the use of ancillary techniques is recommended to improve diagnostic accuracy. Genotyping is nowadays considered the gold standard methodology used in routine diagnostic workflow.

Aim: Evaluate the performance of DNA genotyping in diagnosis of HMs

Principle of methodology: Microsatellite polymorphisms based on short tandem repeats (STRs) are used to diagnose HMs by comparing allelic profiles from maternal decidua and chorionic villi. This analysis determines the parental origin and proportion of alleles in the placenta, providing key genetic information.
This methodology offers clear advantages as it simultaneously determines ploidy and parental origin, distinguishing molar pregnancies from mimics through allele peak analysis.

Discussion: Genotyping is typically straightforward when comparing placental and maternal tissue. However, there are some pitfalls in using molecular genotyping as tissue cross-contamination that might compromise the interpretation.

In rare scenarios, some CHMs are tetraploid but likewise purely androgenetic origin, making them indistinguishable by genotyping. Familial biparental recurrent CHM, linked to maternal-effect mutations in NLRP7 or KHDC3L genes, mimics CHM histologically but retains a diploid biparental genome with p57 loss.

Furthermore, molecular genotyping of placental tissue resulting from pregnancies that contain the donor's egg genome instead of that inherited the recipient mother's genome will present a unique allelic pattern that is not present in the recipient's maternal tissue.
For this reason, correlation with the clinical context is also required and a correlation of several techniques, including morphology assessment, p57 immunohistochemistry and karyotype should always be done to establish an accurate diagnosis.

CO39

LIFE IS LIFE. NEVER STOP TALKING ABOUT LIFE

Jorge Pinheiro[1], Alvim A.[1], Almeida T.[1], Anastácio M.[1], Camara A.[1], Carpalhoso B.[1], Carriço A.[1], Carvalho A.[1], Casalinho L.[1], Costa P.[1], Cunha R.[1], Ervilha C.[1], Figueiredo M.[1], Gomes D.[1], Guerra A.[1], Lopes C.[1], Marrao G.[1], Mendes S.[1], Menezes M.[1], Nunes C.[1], Oliveira A.[1], Oliveira C.[1], Órfão D.[1], Ostapenko N.[1], Pedrosa J.[1], Pinto V.[1], Pires L.[1], Racovica R.[1], Ribeiro Y.[1], Ribeiro da Silva B.[1], Roda S.[1], Rodrigues R.[1], Santos M.[1], Santos L.[1], Silva A.[1], Silva C.[1], Vieira L.[1], Vieira M.[1], Vieira R.[1], Castro Ricardo[1]

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Introduction: Previous studies found that patients distribution data´s charts on Modulab Gold (PD) can be used to detect technical errors in real time, can also assure when it does not affect patients (1), or even to know the CVi for biological magnitudes of a laboratory population (2).
The use of PD is useful only when we can distinguish between a technical error and the music of life (3) of a laboratory´s population´s biological pattern.
Objective: To show PD shifts for serum albumin and total protein, due to the usual biological fluctuation of our Hospital Center´s population.

Material and Methods: It was observed the Modulab Gold PD of our population for serum albumin and total protein, for 5 days.
It was evaluated the internal quality control Levey Jennings charts for serum albumin and total protein.
The serum albumin is dosed with a bromocresol green 600/800 nm bicromatic reading, and total protein with picrate ions 540/660 nm bicromatic reading, on a Beckman Coulter AU 5820.

Results: It was observed a progressive decay at the end of the evaluation period on our population serum albumin´s PD (figure 1), that is replicated at the total protein´s PD (Figure 2).
Stabilized quality control charts were also observed for both serum albumin and total protein at the same period.
The evaluation of the affected patients shows: 38,7% were from Intensive care unit; 29% from emergency; 12,9% from cardiac intensive care unit; 9,7% from polyvalent acute care unit and 9.7% others. With 61% of patients aged more than 80, and 70,9% with acute/cronic infection-like state.

Conclusion: As albumin is 50-65% of total protein and we use different dosing methods for both, this indicates a less likely technical error on both methods, for the observed affected patients on PD.
Since albumin (and total protein) depletion is expected in acutely infected people, and patient characterization indicates that our hospital´s intensive care units follows the same procedures for sample collection and result turnround in most critically infected patients, we can anticipate a small but significant group of patients with this PD pattern during the specific time window period of 5 - 8 am.~

The laboratory professionals that use PD for decision making, must be aware of the biological patterns of its population, that also can depend on their health professional standard procedures.

CO40

ANALYSIS OF INTERCURRENCES DURING BLOOD COLLECTION OF OUTPATIENT PATIENTS AT A CLINICAL ANALYSIS LABORATORY OF A UNIVERSITY HOSPITAL

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Introduction: The collection of biological samples is crucial in the pre-analytical phase, directly affecting result quality. Phlebotomy, a common invasive procedure, involves blood vessel access for diagnosis and treatment. Despite WHO protocol, risks persist. Collection quality impacts patient satisfaction and service loyalty, but limited complication data hinder quality assessment.

Objective: To analyze the type and frequency of intercurrences during blood collection from outpatients in a Clinical Analysis Laboratory of a University Hospital.

Methods: This study, under ethical approval, examined outpatient blood collection intercurrences in a University Hospital laboratory.
Data collection included procedure observation, phlebotomist's reports, and patient interviews, followed by statistical analysis. Categorical variable correlations were assessed using chi-square or Fisher's exact tests (for group size <5). Only significant ($p < 0.05$) associations were reported.

Results: Among 400 collections, 288 (72%) patients experienced intercurrences, totaling 453 occurrences.
The most frequent were pain at needle insertion (48.5%), anxiety/fear (24%), muscle contraction (8.5%), and repuncture (7.8%). Other issues included tourniquet pain (4.5%), hematoma (4.2%), weakness (3.5%), sweating (3.5%), pallor (3%), hand tingling (3%), nausea (1.8%), visual darkening (0.7%), and headache (0.2%). Vasovagal reactions occurred in 13.7% of cases, which occurred separately or in combination, all of them classified as mild and not impacting patients' daily activities.
Correlations between complications and collection characteristics validated practical observations, such as predominance of intercurrences in men, and higher repuncture rate in patients over 50 years old. Collection outside the cubital fossa was associated with pain, pallor, hematoma, repuncture, visual impairment and patients undergoing chemotherapy. In general, high repuncture rates emerged as a neglected quality indicator.

Conclusion: We highlight the need for better phlebotomist training to improve complication management and ensure safer, more effective blood collection.

CO41

SERUM TRYPTASE MEASUREMENT IN CRITICAL SETTING

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Introduction: The diagnosis of anaphylaxis is essentially clinical, and can be supported by measuring the serum level of the enzyme tryptase, which increases in an anaphylactic reaction due to the activation and degranulation of mast cells.
Its transient increase during a severe allergic reaction helps to identify and assess the extent of the reaction, and a high and persistent basal level is an indication of possible systemic mastocytosis.
The sample should be collected up to a maximum of 6 hours after the onset of symptoms (ideally in the first 15-60 minutes): a sudden increase in serum tryptase levels, with regression to baseline values in 24-48 hours, confirms the occurrence of anaphylaxis; however, a normal value does not exclude the diagnosis.

Objectives and Methods: Search the Clinidata® database for the origin of serum tryptase assay requests between 2019 and 2024 in hospital services where there is a higher probability of severe allergic reactions, or where patients with acute episodes of severe allergic reactions are evaluated: Adult Emergency (AE), Pediatric Emergency (PE), Operating Room (OR), Day Hospital (DH), Intensive Care Unit (ICU) and Inpatient.
Identify cases with assay values above the reference value (RV), 11.4 ng/mL, and relate them to the services studied.

Results: Out of a total of 430 requests, the distribution of the origin of the requests was: AE 57.7%, PE 19.1%, OR 7.2%, DH 6.5%, ICU 5.1% and Inpatient 4.4%; the percentage of results above the RV per service was: AE 27.8%, ICU 27.3%, OR 25.8%, PE 18.3%, DH 10.7%; and Inpatient 5.3%; and the percentage of results with a value above the RV per service, relative to the total number of requests was: AE 16%, PE 3.5%, OR 1.9%, ICU 1.4%, DH 0.7% and Inpatient 0.2%.

Discussion: In the Emergency Services, AE and PE, cases occurring in the community are admitted, with the highest number of tryptase requests being verified here. The highest proportion of results above the RV was observed in samples from AE, ICU, OR and PE, which is consistent with the inherent critical environment of these services.
The low positivity in HD and Inpatient Units may justify the optimisation of protocols for requesting this analytical parameter.

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| SESSÃO PRÉMIO MELHOR POSTER

P01

REGULATORY ACTION OF MESENCHYMAL STROMAL CELLS ON NK CELLS IN RHEUMATOID ARTHRITIS

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The increasing knowledge about Mesenchymal Stromal Cells (MSCs) biology has provided the development of new cell therapies, particularly in Rheumatology area of auto-immune etiology. These cells exhibit immunosuppressive properties, having the ability to suppress local inflammation and tissue damage, in a wide variety of diseases, in particular, Rheumatoid Arthritis (AR).

The objective of the study was to analyse the immunomodulatory action of bone marrow derived MSCs, in the ability of peripheral blood NK cells from patients with AR to produce inflammatory cytokines and compare the obtained results with those obtained from healthy individuals. Twelve patients with AR and eight controls were enrolled in this study. We performed co-cultures with peripheral blood mononuclear cells and MSCs during 24 hours. After this period mononuclear cells were activated in vitro with phorbol-12-miristato-13-acetato (PMA) and ionomycin in order to determine the frequency of NK cells producing TNFα or IFNγ, by flow cytometry.

Statistical analysis was performed using GraphPad Prism 6 statistical software for Windows. The values obtained are presented as mean ± standard deviation. The results were statistically analyzed using the Wilcoxon test for paired samples and correlated using Pearson's correlation coefficient.

The results were considered statistically significant when a random error (p) less than or equal to 0.05 ($p \leq 0.05$) was assumed, with a confidence level of 95%. To calculate the percentage of inhibition for the percentage of NK cells producing cytokines under study, as well as their expression, the following equation was used: 100-[(MSCs/MNC)*100].

In the presence of MSCs, it was observed, in both studied groups, a statistically significant decrease in the frequency of NK cells producing TNFα or IFNγ, as well as in the expression of both proteins at single cell level.
It was also noticeable a negative correlation between the relative percentage of inhibition of TNFα or IFNγ expression in NK cells and the disease activity score (DAS) index.
MSCs inhibit, in an efficient way, the ability of cytokine production, namely TNFα and IFNγ by NK cells, and therefore contributing to the decrease of inflammation and AR symptoms.

P02

DETECTION AND IDENTIFICATION OF HAEMOGLOBIN VARIANTS: EXPERIENCE WITH A POINT OF CARE TEST - GAZELLE

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Introduction: Sickle cell disease (SCD) is a major public health problem in Africa. Diagnostic laboratory techniques are expensive, requiring equipment that needs maintenance and trained/qualified technicians to interpret the results. Identification of haemoglobin (Hb) variants requires the use of at least two methods with different separation principles. The lack of rapid and reliable diagnostic methods for screening could result in many avoidable deaths in the affected population. This study was realised as part of the 'Força Saúde' project, which aims to strengthen the alliance between the african and portuguese health systems through the training of human resources.

Objective: The aim of this study was to evaluate the performance of a point-of-care test (POCT) – Gazelle, a cellulose acetate electrophoresis rapid test in the detection and identification of Hb variants, in comparison with the diagnostic methods used in the laboratory, with a view to future implementation of this equipment in African countries.

Methods: The samples (peripheral blood in EDTA) were analysed using the methods used in the laboratory, including the solubility test, haemoglobin isoelectric focusing, high performance liquid chromatography (HPLC) and the Gazelle equipment, according to the manufacturer's instructions. A total of 51 tests were performed, 48 on suspected cases of haemoglobin variants and 3 on samples without haemoglobin variants.

Results: From a total of 51 cases, 48 (94.1 %) were valid and 3 were considered invalid (5.9%) because they were inconclusive and were not included in the analysis. The 48 cases with valid results represented individuals with a mean age of 32 years, of whom 33 (69%) were female and 15 (31%) male.
The Gazelle test correctly identified all Hb S carriers (n=10) and the 4 SCD patients. As expected, since the method is based on cellulose acetate electrophoresis, it failed to distinguish Hb D (n=6) from Hb S (n=10) and Hb C (n=15) from Hb E (n=6).
The specificity was 100% for identifying SCD patients and 84.4% for identifying Hb S carriers. The 3 normal samples for Hb variants were correctly identified.

Conclusion: The POCT Gazelle demonstrated high sensitivity for the detection of Hb S and could be a potential screening tool for the rapid diagnosis of this variant in developing countries where it is highly prevalent and a serious health problem.

P03

MUTATIONS IN THE FACTOR V GENE AND ACTIVATED PROTEIN C RESISTANCE

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Activated protein C resistance is a hereditary or acquired condition that results in the inability of protein C to inactivate clotting factor Va, predisposing the individual to thrombosis [1].
Among hypercoagulable conditions, mutations in the factor V gene are the most common. The R506Q mutation (Factor V Leiden) results in the substitution of arginine for glutamine at position 506 of the protein, making it resistant to inactivation by activated protein C. The H1299R variant involves a single nucleotide polymorphism at position 4070 of the Factor V gene, where adenine is replaced by guanine.

This nucleotide change leads to the replacement of histidine for arginine at position 1299 of the Factor V protein [2,3].
This study aims to identify the prevalence of R506Q and H1299R mutations in patients referred to Immune-thrombophilia consultation, as well as to evaluate the association between these mutations, activated protein C resistance, spontaneous abortions and thromboembolism.

Methods: Genetic study: DNA extraction was performed with the QIAmp DNA Blood Mini Kit (Qiagen) using the QIAcube automatic extraction equipment. Variants R506, H1299R (haplotype H2) and Y1702C were investigated using the AnyplexTM II Thrombosis SNP Panel ASAY by real-time PCR.
Activated protein C resistance: It was performed with the Staclot® APC-R kit on the STA R Max® equipment.

Results: Of the 111 patients evaluated, 28 presented mutations in the Factor V gene, but only 12 exhibited activated protein C resistance. The analysis of cases with activated protein C resistance revealed the presence of the R506Q variant in all cases. The remaining 16 cases presented the H1299R variant (H2 haplotype) in the Factor V gene (14 heterozygous and 2 homozygous). Among the cases with the H1299R variant are all women with a mutation in the Factor V gene referred to this consultation due to spontaneous abortions (totaling 5 patients).

Conclusions: Approximately 25% of patients presented mutations in the Factor V gene. The R506Q mutation is the main cause of activated protein C resistance in the studied patients.
Although the H1299R variant is present in a significant number of patients, it was not responsible for activated protein C resistance. However, its presence may be associated with other risks and should not be disregarded, as it appears to be linked to cases of spontaneous abortions.

P04

B/T MIXED-PHENOTYPE ACUTE LEUKEMIA: A CASE REPORT

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Introduction: Mixed phenotype acute leukemia (MPAL) is a rare and heterogeneous group of acute leukemias with poor prognosis, characterized by distinct blast subpopulations with different immunophenotypes or biphenotypic blast population co-expressing markets of different lineages. Immunophenotyping by multiparametric flow cytometry (FCM) is essential for establishing the diagnosis.

Case report: We present a case of a 15-year-old boy recovering from COVID-19, with no significant medical history. He complained of abdominal pain and fever lasting for 5 days. Physical examination revealed diffuse abdominal tenderness and hepatomegaly.
Laboratory findings included leukocytosis (17,030/uL) with neutrophilia (8,870/uL), lymphocytosis (5,010/uL) and monocytosis (2,330/uL), low platelet count (82,000/uL), elevated lactate
dehydrogenase (658 mg/dL) and high C-reactive protein (96 mg/dL). CT-scan revealed hepatosplenomegaly and multiple abdominal and mediastinal adenomegalies. The peripheral blood smear showed blast cells.
Bone marrow aspirate was normocellular with 38% blast cells. FCM analysis revealed a single population of lymphoid blasts, accounting for 37% of the total cells. The blasts expressed cCD3, CD19, CD79a, CD10, CD2, CD5, CD8, CD38, CD7 and CD13. They showed weak positivity for CD21 and CD123 and were negative for MPO, CD1a, CD3, CD4, CD9, CD11b, CD20, cCD22, CD24, CD33, CD34, CD117, TdT, sIgM, Kappa and Lambda. The central nervous system was free of disease, but testicular involvement was confirmed. Cytogenetic analysis showed a normal karyotype, and molecular studies revealed no high-risk alterations, but detected deletions in CDKN2A/B genes.

Discussion: The diagnosis of MPAL is based on the presence of a blast population exceeding 20% of the total cell population, exhibiting antigenic expression of more than one lineage.

According to the World Health Organization (WHO) criteria for assigning multiple lineages to a single blast population, the patient's blast cells exhibited co-expression of T-cell (cCD3) and B-cell (CD19, CD10, and CD79a) antigens, leading to the diagnosis of biphenotypic B/T MPAL, one of the least common subtypes within the MPAL category.

Conclusions: MPAL is a rare entity and FCM immunophenotyping with a comprehensive antibody panel is essential for its diagnosis.
WHO-defined lineage-specific markers must be included in the standard immunophenotyping panel for acute leukemia to prevent underdiagnosis of MPAL cases.

P05

T CELL CLONALITY IN THE DIAGNOSIS AND FOLLOW-UP OF T-CLPD: THE ROLE OF THE TRBC1 ANTIBODY

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Introduction: T cell chronic lymphoproliferative disorders (T-CLPD) are uncommon lymphoid malignancies derived from post-thymic T cells, that comprises an heterogeneous group of entities with variable clinical behavior and biologic features.
Compared to B cell chronic lymphoproliferative disorders, whereas the assessment of the cell clonality is well established, the diagnosis of T-CLPD by flow cytometry (FC) is often challenging, depending on the presence of immunophenotypic aberrations.
The current gold standard techniques are the PCR of T-cell receptor (TCR) gene rearrangement and the FC TCR Variable Beta Region (TCRVB) repertoire, which are highly complex, expensive, time-consuming, requires skilled personnel and are not routinely available in many diagnostic laboratories.
Lately, a monoclonal antibody specific for TCR β chain constant region 1 (TRBC1) (JOVI.1 clone) has been used to easily and quickly evaluate T cell clonality in αβ TCR-positive T cell disorders. The inclusion of the TRBC1 in the same analysis tube of the core T cell antigens, ideally on a 8 to 10 color FC set up, allows the distinction of different T cell subsets and the optimal separation of neoplastic cells from the reactive background ones.
To show the utility of the inclusion of the TRBC1 in T-CLPD FC diagnosis we decided to present an example of a T-CLPD: T cell large granular lymphocytic leukemia (T-LGLL).

Clinical Report: A 54-year-old female with a history of myelodysplastic syndrome and T-LGLL showed a worsening anemia. The FC immunophenotyping study revealed 19.40% of normal T cells and 7.31% pathological T cells with the following immunophenotype: CD2+ CD3+ CD4- CD5+dim CD7+ CD8+ CD26- and TRBC1+, compatible with persistence of the T-LGLL. The pathological cells presented a monotypic profile of TRBC1 (100% TRCB1+), whereas the normal T cells showed a polytypic/bimodal distribution.

Discussion / Conclusion: With the implementation of TRCB1 in the laboratory routine it was possible to verify the presence and persistence of specific immunophenotypic features characteristics of T-LGLL cells, distinguishing them from reactive expansions and small T-cells clones of uncertain significance. In this case, the use of the TRBC1 was useful to identify clonality and thereafter the presence of pathological cells (CD3+CD8+), even in a lower percentage.

Keywords: T cells, T-CLPD, Flow Cytometry, TRBC1

P06

BROADENING THE SCOPE OF COMPLETE BLOOD COUNT: THE POTENTIAL OF RET-HE AND HYPO-HE IN THE STUDY OF CERTAIN TYPES OF ANEMIA

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Introduction: Parameters like reticulocyte haemoglobin (Hb) content (Ret-He) and the percentage of erythrocytes with low Hb content (%Hypo-He) have been described as useful in the study of certain types of anemia and in the early monitoring of response to iron and erythropoietin therapy.

Aim: Evaluate Ret-He and Hypo-He, considering their role in anemia profiling and the advantages of their inclusion in the complete blood count (CBC).

Materials and Methods: Retrospective cross-sectional study conducted with data collected between May and December 2024 from individuals ≥18 years old with CBC, reticulocyte count, iron studies, and C-reactive protein (CRP) requests. Hematological and biochemical analyses were performed using the Sysmex® XN-3100 and Abbott Alinity® analysers, respectively. Descriptive statistics, normality testing (Shapiro-Wilk), Spearman correlation analyses, and Mann-Whitney tests were performed in SPSS v20.02. Cut-off values were set at ferritin <30 µg/L and <15 µg/L, Ret-He ≥29 pg, and %Hypo-He <2.5%.

Results: A total of 495 CBCs were analysed (57% female, 43% male, both with a median age of 75 years). Statistically significant differences were observed in the median values of Ret-He and %Hypo-He between groups with ferritin <15 µg/L and >30 µg/L ($p < 0.001$), with Ret-He values increasing and Hypo-He values decreasing as ferritin levels increased. Among individuals with Ret-He ≥29 pg, 65% had ferritin ≥30 µg/L, while 5% had ferritin <30 µg/L. In those with Ret-He <29 pg, 6% had ferritin <30 µg/L, and 24% had ferritin ≥30 µg/L, 91% of the latter had CRP >0.5 mg/dL. In this group, lowering the Ret-He cut-off to <26 pg reduced discordant cases to 13%.
Regarding %Hypo-He, 71% of individuals with ferritin ≥30 µg/L had Hypo-He <2.5%, whereas 18% had values ≥2.5%.

Discussion and Conclusions: Ret-He and Hypo-He proved relevance when assessed alongside the other biomarkers, particularly in identifying possible early responses to iron therapy and in the potential detection of iron deficiency in anemia of chronic disease. Defining cut-off values tailored to the population may optimize outcomes.
Given the sample median age, other markers should be considered for a more detailed study. Our findings reinforce the usefulness of these parameters for a more comprehensive erythropoiesis assessment upon reticulocyte testing while highlighting the need to raise awareness of their proper integration into clinical practice.

P07

MILIARY TUBERCULOSIS: RARE BUT PRESENT!

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Introduction: Miliary Tuberculosis (MTB) is a rare form of Tuberculosis (TB), which results from the lymphohematogenous dissemination of Mycobacterium tuberculosis (MT).
Clinical manifestations can be diverse according to the organs involved. Males are the most affected. Diagnosis is highly probable in the presence of a diffuse miliary infiltrate on chest X-ray, but bacteriological confirmation is essential for monitoring the disease and knowing the antimicrobial susceptibility profile (AST).

Case description: Male, 51 years old, Romanian, living in Santarém, with ethanol habits and active smoking for 30 years. After 2 months of odynophagia and dysphagia, he was admitted for a microbiopsy and tracheotomy due to a suspected neoplasm in the laryngopharynx. During hospitalization, a chest X-ray showed marked bilateral infiltrates with a miliary appearance. On suspicion of MTB, sputum was collected for microbiological culture and nucleic acid amplification techniques (PCR), as well as blood cultures. He developed sepsis with multiple organ failure. The following laboratory results were obtained: #1 Sputum: Ziehl-Neelsen stain, positive for acid-fast bacilli (BAAR); PCR positive for MT; negative for mutations associated with rifampicin (rpoB gene) and isoniazid (inhaA and KatG gene) and cultural for mycobacteria, positive; #2 Blood culture: positive for MT; #3 AST from the culture confirmed sensitivity to all antibacillars instituted; #4 Biopsy revealed a granuloma without evidence of neoplasia and the presence of BAAR. Today, the patient is in the ICU. There is still no favorable evolution and the chest CT scan still shows severe diffuse bilateral lung involvement.

Discussion: This case is intended to draw attention to the following: #1 MTB is rare, but it does exist! The initial suspicion of neoplasia would be a more likely diagnosis, given the rarity of MTB (2%). The patient has risk factors: gender, country of origin and area of residence with a higher incidence of TB, smoking and ethanol habits. #2 Since TB is a slow-onset disease, diagnosis is often late, which explains the high mortality and morbidity rate. The patient is still in critical condition. #3 An assertive laboratory approach was essential in reducing the risk of hospital transmission to other patients and the healthcare professionals involved. The rapid laboratory response through positive bacilloscopy and PCR testing enabled effective therapeutic decisions and public health measures to be taken.

P08

BERGEYELLA ZOOHELCUM SÉPSIS IN HEMODYALIS PATIENT

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Introduction: Bergeyella zoohelcum is a Gram-negative, aerobic, non-motile rod from the Flavobacterium complex, rarely isolated in humans. This is the case of an 83-year-old retired female patient who developed sepsis caused by this bacterium.

Objective: To analyse the laboratory identification of Bergeyella zoohelcum in a hemodialysis patient.

Case Presentation: The patient had been undergoing hemodialysis since 2016 without complications. On October 4, 2023, during a session, she developed shivering without other symptoms. She had a cat scratch on her left lower limb, which had been present for three days, with no signs of local inflammation.
The patient lived with three unvaccinated cats and two rabies-vaccinated dogs. She was treated with paracetamol, and blood cultures were collected. Laboratory tests showed a leukocyte count of 15,940/µl and C-reactive protein of 114.9 mg/dl.
Blood cultures were incubated in a BioMérieux®BactAlert system and became positive after two days. A subculture was done on Blood Agar, and a Gram slide revealed Gram-negative rods.

After 18 hours of incubation at 36°C, Bergeyella zoohelcum was isolated and identified by Bruker®MALDI-TOF. The antibiogram with BioMérieux®E-test showed susceptibility to amoxicillin, amoxicillin/clavulanic acid, meropenem, and ciprofloxacin, using the EUCAST® species non-specific breakpoints.

Discussion: Bergeyella zoohelcum is commonly found in the upper respiratory tracts of pets but rarely infects humans. These infections typically result from bites, scratches, or prolonged contact with animals. It is an opportunistic pathogen that requires an entry point, such as a skin breach. It can cause cellulitis, abscesses, tenosynovitis, sepsis, pneumonia, and meningitis. In this case, the patient developed sepsis and was treated with iv ceftazidime for two weeks. Though ceftazidime was not tested in vitro due to the unavailability of the E-test, B. zoohelcum infections are usually sensitive to beta-lactams and quinolones. The patient fully recovered, and follow-up blood cultures were negative.

Conclusion: Opportunistic, low-virulence bacteria should be considered potential pathogens in immunocompromised patients.
This rare case highlights the importance of detailed patient history and the role of MALDI-TOF technology for precise, rapid pathogen identification, enabling an accurate clinical diagnosis.

P09

A SILENT DIAGNOSIS: MULTIDRUG-RESISTANT TUBERCULOSIS IN AN OCCUPATIONAL LUNG DISEASE SUSPECT

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Introduction: Pneumoconioses result from chronic inhalation of inorganic dust, primarily occupational. Initially asymptomatic, they increase lung cancer and respiratory disease risks.
Pulmonary tuberculosis (TB), caused by Mycobacterium tuberculosis, is often insidious, with nonspecific symptoms that delay diagnosis. In such cases, insidious infections like TB should be considered, even when occupational lung disease seems more likely.

Case Report: A 63-year-old hypertensive male with a 30 pack-year smoking history has always worked in construction, with long-term exposure to stone and iron dust. In May 2024, he consulted his primary care physician due to complaints of persistent cough. Chest X-ray and CT scan showed "(...) calcified lymphadenopathy and peri-lymphatic pulmonary micronodules, suggesting silicosis.
Additionally, a 35x27mm soft tissue mass with long spicules extending to the pleura raised suspicion of a neoformative lesion (...).". He was then referred to a pulmonology specialist at a hospital for further evaluation.
There, a full diagnostic workup was initiated, including a bronchoscopy with bronchoalveolar lavage performed on September 17th.
The next day, an acid-fast bacillus (AFB) smear was negative, but cultures turned positive on October 1st in MGIT and Löwenstein-Jensen media, identifying M. tuberculosis complex. He began 1st-line TB treatment HREZ [Isoniazid (H), Rifampicin (R), Ethambutol (E), Pyrazinamide (Z)] the following day. Five days later, drug susceptibility testing identified resistance to Isoniazid and Rifampicin, detecting rpoB D516V and katG S315T mutations.

The patient was hospitalized on October 11th to initiate 2nd-line TB treatment BLMZ [Bedaquiline (B), Linezolid (L), Moxifloxacin (M), Pyrazinamide (Z)] and was placed in respiratory isolation. Nine days later, phenotypic confirmation of multidrug resistance and additional Pyrazinamide resistance were obtained. Following a favorable clinical response and nearly after two weeks of hospitalization, the patient was discharged on October 24th.
This decision was made by consensus with medical team and National Mycobacteria Reference Center, with outpatient follow-up at Pulmonary Diagnostic Center.

Discussion: Early identification of M. tuberculosis and resistance markers enabled timely treatment adjustments, improving outcomes. Clinician-laboratory collaboration was key to guiding therapeutic decisions, reinforcing the value of a multidisciplinary approach in complex cases.

P10

ADJUNCTIVE HYPERBARIC OXYGEN THERAPY IN CHRONIC OSTEOMYELITIS: PATHOGEN PREVALENCE AND CLINICAL EFFICACY

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Introduction: Chronic osteomyelitis is defined as the persistence or recurrence of infection despite adequate surgical debridement and antibiotic therapy. Although these remain the cornerstones of treatment, hyperbaric oxygen therapy (HBOT) has been used as an adjunctive option and is currently classified as an AHA Class IIa recommendation. However, no randomized clinical trials have been conducted, and recent data on its efficacy remain scarce. This study aimed to report our centre's experience and analyze the prevalence of infectious agents associated with chronic osteomyelitis.

Methods: We conducted a retrospective observational study of all patients aged 17 years or older with a confirmed diagnosis of chronic osteomyelitis who underwent HBOT between 2006 and 2024. Data were collected through a review of medical records. Patients with acute osteomyelitis, concomitant diabetic foot infections, or incomplete data were excluded.

Results: A total of 87 patients underwent HBOT. Before treatment, all patients had received antibiotics (75% targeted), and 81% had undergone surgical debridement. The most commonly affected bone was the femur, followed by the tibia, with foreign material present in approximately 20% of cases.
The most frequently identified pathogen was *Staphylococcus aureus* (56.1%), with 28.8% methicillin-sensitive *S. aureus* (MSSA) and 27.3% methicillin-resistant *S. aureus* (MRSA). Other prevalent pathogens included *Pseudomonas aeruginosa* (12%), *Escherichia coli* (4.5%), and *Enterococcus faecalis* (4.5%).
During HBOT, 99% of patients continued antibiotic therapy. At six-month follow-up, 82% of patients remained disease-free.

Conclusion: HBOT appears to be a safe and effective adjunctive therapy, providing temporary positive outcomes in most patients and improving their quality of life. However, controlled studies are needed to evaluate its impact on long-term prognosis further.

P11

NOT ALL IS AS IT SEEMS: ALIARCOBACTER BUTZLERI A CAMPYLOBACTER-LIKE EMERGING BACTERIA CAUSING ACUTE GASTROENTERITIS

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Introduction: In 2002, the International Commission on Microbiological Specifications for Food designated Aliarcobacter butzleri as an emerging enteropathogen, classifying it as a "serious hazard to human health". Since then, epidemiologic studies, outbreak reports, and case reports increasingly evidence this bacterium as a food and waterborne pathogen associated with gastrointestinal illness, mostly manifested by a more persistent watery diarrhoea, yet less bloody, compared to that caused by taxonomically related Campylobacter jejuni.
However A. butzleri incidence may be underestimated since routine clinical laboratories are biased toward the detection of phenotypically similar Campylobacter spp.

Case Report: A 4-year old male child with beta thalassemia and slow weight gain presented with a 1-week history of reduced oral intake and 2 days persistent vomiting and mild fever. On admission, he was moderately dehydrated and tachycardic. Laboratory tests showed leucocytosis, neutrophilia, thrombocytosis, and elevated CRP (64.78 mg/L). The symptoms progressed to a watery non-bloody diarrhoea (6-7 stools daily). Stool tests were negative for enteric viruses and common enteropathogenic bacteria; however, Campylosel agar (bioMérieux, Marcy-l'Étoile, France) yielded small, greyish glistening colonies, resembling Campylobacter morphology, after 4 days of incubation at 42°C under microaerophilic atmosphere.
Unexpectedly, MALDI-TOF MS identified the isolate as A. butzleri, with MICs of 0.094 mg/L for ciprofloxacin, 3 mg/L for erythromycin, and 2 mg/L for tetracycline determined by concentration gradient strip technique. The patient improved significantly after 5 days of intravenous fluid therapy and supportive care, without requiring antibiotics.

Discussion: While not optimal, Campylosel agar, a Campylobacter selective agar, appear to support the growth of A. butzleri.
In routine clinical laboratories which do not perform more than presumptive identification of Campylobacter spp., based on phenotypic characteristics that overlap between these organism, A. butzleri may be misidentified as Campylobacter spp. without the use of MALDI-TOF MS.
This underscores the need for optimize isolation procedures and advanced identification methods to improve surveillance and ensure timely, appropriate management of infections caused by emerging pathogens.

P12

CTX/ BAP: UTILITY ON OSTEOLYTIC LESIONS AND PROGRESSION OF MULTIPLE MYELOMA

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Introduction: Multiple myeloma (MM) is a B-cell malignancy characterized by the production and accumulation of clonal activated plasma cells in bone marrow, which are able to develop bone lytic lesions, often detected at diagnosis by imaging. Bone markers, such as C-terminal telopeptide of collagen type 1 (CTX), a marker for bone destruction, and bone-specific alkaline phosphatase (BAP), a marker for bone formation, offer potential for earlier detection. An increased CTX/BAP ratio may suggest the presence of bone destruction and may be useful in the diagnosis of MM and providing distinction from diseases such as monoclonal gammopathy of undetermined significance (MGUS) and other hematological pathologies (HP).

Objectives: Measure serum CTX and BAP levels, as well as the CTX/BAP ratio, in MM patients with osteolytic lesions and evaluate differences with MGUS, HP, and control (CTL) groups.

Materials and Methods: Serum samples from 154 patients (42 MM, 20 MGUS, 43 HP, and 49 CTL) collected between 2019 and 2024 were analyzed.
CTX was measured via electrochemiluminescence (cobas e601, Roche Diagnostics®; limit of detection: 0.01 µg/L), while BAP was measured using chemiluminescence (iSYS, Immunodiagnosticsystems®; limit of detection: 1 µg/L).

Results: MM patients exhibited higher CTX levels (mean: 0.61 µg/L) and lower BAP levels (mean: 13.98 µg/L) than other groups, resulting in a significantly elevated CTX/BAP ratio (mean: 0.06). MGUS, HP, and CTL groups showed lower CTX/BAP ratios, averaging 0.02. Kruskal-Wallis test confirmed significant differences between MM and other groups ($p < 0.05$). ROC curve analysis showed an area under the curve (AUC) of 0.72 for the CTX/BAP ratio, with a cutoff value of 0.029.

Discussion/Conclusion: CTX levels and CTX/BAP ratio effectively differentiate MM from MGUS and other conditions, reflecting bone destruction associated with osteolytic lesions.
The CTX/BAP ratio demonstrates moderate diagnostic ability (AUC: 0.72) and potential for disease staging and monitoring. Longitudinal studies are needed to validate these findings, particularly in populations with age-related bone conditions. This study highlights the utility of CTX and CTX/BAP ratio in improving MM management.

P13

CLINICAL TO FINANCIAL: HOW TO MANAGE THE LDL MEASUREMENT?

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Introduction: Low-density lipoprotein cholesterol (LDL) is associated with atherosclerotic cardiovascular disease (ASCVD). Lowering LDL is the first lipid target for preventing ASCVD. The decision on initiation of LDL lowering drug therapy or its adjustment is based on the risk of cardiovascular events and on the level of LDL, therefore an accurate determination of LDL is highly important. The gold standard method for determining LDL is β-quantification, a time-consuming and expensive procedure based on ultracentrifugation. Other direct methods using chemical products are also time-consuming and expensive and lack standardization. The Friedewald formula (1972) has been used to estimate LDL, although with some limitations, especially in cases of hypertriglyceridemia.

Recently 2 new equations were developed to improve accuracy of LDL estimation: Martin-Hopkins (2013) and Sampson (2020). However, there is not enough data to establish superiority of one equation over the other.

Aim: The aim of this study is to assess the clinical and financial impact of switching between these equations, comparing with the direct measurement of LDL.

Methods: Direct LDL was measured in serum by a photometric method using liquid selective detergent (Alinity C). Data for individuals who had a complete lipid panel ordered clinically during the year 2024 was analysed. The 3 formulas for estimated LDL were applied.
Calculated LDL were compared with direct LDL according to the Spearman correlation and the Friedman test ($p<0.05$).

Results: The Spearman correlation coefficients between direct LDL and the formulas were, 0.959, 0.967 and 0.966, respectively, indicating a strong positive correlation. Friedman's test revealed statistically significant difference between the methods, suggesting that the LDL values estimated are not identical.
The cost of 2024 direct LDL measurement was around 10 thousand euros. If we had applied the formulas in these cases, we would only have to measure direct LDL in cases where TG≥400mg/dL, having obtained only a maximum cost of 76 euros.

Conclusion: The formulas had a good applicability, exhibiting a good correlation with the direct LDL. However, there are differences in the median values. Switching between different methods could have clinical implications for many patients.
Therefore, their follow-up should be done by the same method. Concerning the financial part, the savings are notable when using the calculated LDL and should be considered.

P14

DETERMINATION OF PSA(S) AND PROSTATE VOLUME: ANALYTICAL AND CLINICAL PERFORMANCE IN PROSTATE CANCER

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Introduction: Prostate Cancer (PCa) screening with PSA has been scrutinized in recent years due to documented overdiagnosis and its relatively low diagnostic specificity, highlighting the need for new biomarkers that add predictive value to patients at the time of diagnosis. The Prostate Health Index (PHi) (Beckman Coulter, BC) combines the results of 3 quantitative immunoassays of kallikreins, total PSA (tPSA), percent free PSA (%fPSA), and [-2]proPSA (p2PSA) into a single numeric score (PHi score = p2PSA/fPSA × √tPSA).
Here, we aimed to compare BC serum tPSA measurement with currently used method and evaluate the clinical accuracy of PHi and Phi density to discriminate malignant from benign prostatic disease.

Methods: Blood samples from 140 men referred to the Urology consultation were used to measure matched tPSA, %fPSA and p2PSA levels using Access®2 (BC) and Cobas®801 (Roche). Comparison of tPSA between methods included Passing-Bablok regression and Bland-Altman analysis. Multiparametric magnetic resonance imaging of the prostate was used to calculate the prostate volume (PV). Non-parametric tests were used to compare central tendency measures between malignant and benign cases. PSA and PHi density were calculated by dividing the serum PSA/PV. Receiver Operating Characteristic (ROC) curves and AUC were used to assess the accuracy of PSAs to predict malignancy in prostatic biopsy.

Results: A strong correlation was found between the two instruments (r=0.987), with 97.5% agreement in tPSA values.
Passing-Bablok regression (Cusum test, P=0.16) confirmed absence of significant deviation from linearity, whereas Bland-Altman analysis a reduced mean difference (0.4010, 95%CI -0.462 to 1.264).PCa patients presented with significantly lower prostate volume, while tPSA and %fPSA were more elevated in benign conditions.
PSA density (PSAD) and PHi were significantly higher in malignant cases. ROC analysis revealed that the AUC for both PHiD and PHi was approximately 0.80, indicating high diagnostic accuracy. tPSA and PSAD had AUC around 60%.

Conclusion: PHi outperformed tPSA, confirming the superior precision in PCa detection as confirmed by biopsy. Phi and PHiD are more robust markers for detecting malignancy in men suspicious for prostatic disease.
This also apllies to expanded data beyond "gray zone" of negative DRE tPSA values. Further studies should determine clinical threshold of PHi and PHiD values in order to incorporate them into risk calculators.

P15

DEVELOPMENT OF A CENOBAMATE QUANTIFICATION METHOD USING AN EXISTING LC-MS/MS ASSAY FOR ANTIEPILEPTIC DRUGS: A NEW (RE)EVOLUTION

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Introduction: Therapeutic drug monitoring (TDM) plays a crucial role in optimizing the clinical management of epilepsy by measuring serum concentrations (SC) of antiepileptic drugs (AEDs). Due to the wide variety of AEDs and their variable SCs, monitoring can be complex. Additionally, pharmacokinetic (PK) interactions between these drugs further complicate treatment. Therefore, accurate quantitative methods are essential for investigating the PK and pharmacodynamic effects of AEDs administration, ultimately improving patient care.
Cenobamate (CNB), approved by the EMA in 2021 for adults with refractory focal seizures, shows strong clinical efficacy. Due to its potential drug interactions in coadministration, commonly used in these patients, dose adjustments are essential for safe and effective treatment.

Objective: This work aimed to expand the simultaneous measurement of several AEDs in a previously implemented liquid chromatography tandem mass spectrometry (LC-MS/MS) method, by incorporating CNB quantification in serum, improving the existing TDM.

Materials and methods: The LC-MS/MS system used was a UPLC System/Mass triple quadrupole detector. Chromatographic separation used a C18, 3µm 2.0x50mm, column (VDSpher PUR 100). AEDs calibrators, internal standards and controls were commercially available (Recipe) and the added CNB materials were purchased from Alsachim. A convenient mobile phase gradient was established with positive electrospray ionization. The 2 specific transitions for the CNB were 268,5>198,3 and 268,5>155,3 and the calibration established, ranged from 1.49 to 29.77 mg/L. The sample run time was 10´ and the injection volume 35µL. Analytical indicators and assessment of patient´s TDM outcomes were analyzed.

Results: The linearity, LOD and LOQ were specifically established within the existing analytical method. Calibration curves showed r2<0.996. Intraassay and interassay precision were <5% and <10%, respectively. Considering PKs, a good agreement was obtained between dose and SC.

Conclusions: This assay proved to be suitable for quantifying CNB and other concomitant AEDs, clinically and analytically. The addition of CNB enhances the method's applicability for an increasingly used drug, ensuring accurate and specific measurement while preventing unwanted drug interactions. This contributes decisively to minimize adverse effects and optimize patient management, reinforcing the importance of precise TDM for improved clinical outcomes.

P16

ARE WE SELECTING THE RIGHT ANALYTICAL PERFORMANCE SPECIFICATIONS (APS) FOR OUR LABORATORY? WILL WE BE ABLE TO ACCOMPLISH THAT SPECIFICATION?

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Background: Clinical laboratories provide essential information for medical decisions related to patient management. This information must be as accurate as possible, with any errors remaining within an acceptable range for medical use. Analytical performance specifications (APS) are used to quantitatively evaluate assay performance, aiming to deliver data suitable for clinical patient care. A consensus conference held in Milan in 2014 recommended three models for establishing APS: 1) direct and indirect outcome studies, 2) biological variation, and 3) state-of-the-art practices. Laboratories should pursue the higher models in the hierarchy whenever data is available and when the current method performance allows for these goals.

Aim: The APS chosen by our laboratory for the serum glucose analyte is model 2 – biological variation (BV), which has a desirable specification of 6.1% (reviewed in January 2025). We aim to determine whether our selected APS is being met.

Methods: We applied the selection algorithm for APS proposed by the AEFA organization. After reviewing the existing APS in the literature, we selected candidate APS - BV and assessed the overall difficulty of achieving this objective by consulting state-of-the-art graphics (showing the percentage of labs meeting 100% of the selected APS). For BV minimum (9.2%), the overall difficulty is medium; for BV desirable (6.1%), it is high; and for BV optimal (3.1%), it is very high. We then calculated the maximum limit of error (ML) using the average error (Xm), the t-student test (t), the number of months of external quality assessment (EQA) (n), and the standard deviation (SD). The formulas used were Xm - (t(95, n - 1)) * SD for the lower limit and Xm + (t(95, n - 1)) * SD for the upper limit. Xm was derived from 12 months of EQA (2024).

The coefficient of variation (CV) was obtained from six months of internal quality control (first semester of 2024). We calculated our total error (TE) using the formula: 1.65 * CV + |Xm|.

Results: The ML was 3.43%, and the TE was 2.86%. By comparing our selected APS (6.1%) with the ML (3.43%) and the TE (2.86%), we conclude that we are meeting the chosen APS, as both the ML and TE are below 6.1%. However, the optimal BV remains unattainable since the ML exceeds 3.1%.

Conclusion: This method for selecting APS helps the laboratory evaluate whether the selected APS is being achieved and provides tools for improvement.

P17

ARE VIRAL INFECTIONS KEY INDUCERS OF MYELIN OLIGODENDROCYTE GLYCOPROTEIN-ASSOCIATED DISORDER? FOCUS IN ACUTE DISSEMINATED ENCEPHALOMYELITIS

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Introduction: Myelin-oligodendrocyte glycoprotein antibody-associated disease (MOGAD) is an autoimmune pathology, defined as an inflammatory demyelinating disease of the central nervous system.
The most common clinical manifestations of MOGAD include acute disseminated encephalomyelitis (ADEM), bilateral optic neuritis (ON) and transverse myelitis (TM). ADEM with or without concomitant involvement of the optic nerve is most prevalent in children, while ON and TM tend to be more frequent in adults. The association of an infectious prodrome in most children with ADEM and anti-MOG IgG antibodies (ab-MOG) suggests that an infection could trigger an immune response that leads to the production of these antibodies, often follow infections such as Influenza, Epstein-Barr, Herpes simplex, among others.

Aim: Analysis of positive results for ab-MOG with ADEM, in the pediatric population, and to evaluate a possible relationship with viral infections.

Methods: Between 2019 and 2024, 112 serum samples of the pediatric population (0-17 years) were tested for ab-MOG using the Fixed cell-based assay - full-length MOG technique (Euroimmun®). Samples positive for ab-MOG (RV <1:10) with ADEM were subjected to complementary analysis of viral serology results from the time of diagnosis aiming to investigate potential correlations. This analysis was performed using Microsoft Excel®.

Results: The median age of children diagnosed with ADEM in the present study was 6 years. Analysis of the data showed that the majority of the samples (79%) were negative for ab-MOG. Of the 112 children with suspected MOGAD, only 24 (21%) had titres > 1:10 for ab-MOG. Among these positive samples, 18 children showed clinical signs compatible with ADEM, of which 11 children (61%) had recent viral infections. The most prevalent viruses in this study were Mycoplasma, Enterovirus, Adenovirus, Herpes simplex and Epstein-Barr.

Conclusion: This study found that the majority of children (61%) had serologies compatible with the presence of recent viral infections, while 5 had previous viral infections.

Despite the small sample size, the results align with literature showing a correlation between infections and the manifestation of ADEM. Unlike other studies, Enterovirus was the most prevalent virus in the infections that triggered ADEM, being the most frequently identified in the cases analyze.

| POSTERS EM EXIBIÇÃO

P18

THE MYSTERIOUS RING-SHAPED NUCLEUS: ARTIFICIAL INTELLIGENCE AS AN ALLY IN EOSINOPHIL DIFFERENTIATION – A CASE REPORT

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Background: This case involves a 59-year-old male patient who was referred to clinical hematology due to a slight increase in total WBC count with no apparent clinical cause. A full blood count was performed using the Sysmex XN-1000, which misclassified eosinophils as neutrophils. The differential count was corrected by microscopy, where an eosinophil with a ring-shaped nucleus was identified. Artificial intelligence (AI)-based image processing, using a custom-trained algorithm, allows high-resolution assessment of nuclear morphology to investigate the potential causes of eosinophil misclassification by the hematology analyzer.

Objective: Pilot study demonstrating how AI can enhance eosinophil detection.
Materials and Methods: Using eosinophil images from CellaVision, AI-based image processing analysis enabled high-precision identification of eosinophils and the detection of morphological changes. AI-based image processing analysis was trained using digital eosinophil images from 25 patients, where eosinophil counts were accurately measured by the cytometer and confirmed by microscopy.

Results: In this patient, the eosinophil count was 0.9% (WBC: 7.9 × 10^3/µL) in the WBC scattergram, without triggering any flag for peripheral blood smear (PBS) review. PBS evaluation was performed only due to an anemia-related flag, leading to a corrected eosinophil count of 12%, which also revealed an eosinophil with a ring-shaped nucleus. AI-based image processing analysis further identified nuclei eosinophils exhibiting unusual morphology, hypodense chromatin (quantified by pixel intensity) and atypical granules.

Discussion/Conclusion: The identification of an eosinophil with a ring-shaped nucleus, alongside cytometer count misclassification prompted further investigation of other morphological changes. AI-based image analysis detected morphological alterations in eosinophils, which were potential causes of their misclassification. These alterations may indicate a potential eosinophil dysplasia, which could hypothetically be related to the misclassification in the cytometer count.

Further studies with additional cases are necessary to refine the AI-based image and assess its applicability in routine diagnostics. AI has the potential to revolutionize hematologic diagnostics, ensuring more accurate differential counts and supporting clinical decision.

P19

EVALUATION OF A POINT-OF-CARE TESTING EQUIPMENT FOR FULL BLOOD COUNT

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Introduction: Our Local Unit of Health (ULS) includes 18 primary care centres and 2 hospitals which attends 142974 residents, 33% of whom are elderly. Many of them live in nursing homes or alone, in 14 municipalities with difficult-to-access roads and a deficient public transport network.
ULS's central laboratory is developing a project to install Point-of-Care Testing (POCT) devices across primary care centres to make diagnostic tools more accessible, faster, and closer to the population, decreasing the demand for hospitals.
The socioeconomic condition of this population prompt many cases of anemia which are associated with isolation, malnutrition, chronic diseases, and polymedication. For this reason, we evaluated a fully automated system, HemoScreen™, for full blood counts, which is FDA-approved and IVDR-certified.

Objective: Evaluate a POCT for Full Blood Count

Material and methods: Sample: 29 anonymized whole blood EDTA samples XN3100™, Sysmex™: haemoglobin (sodium lauryl sulphate), leukocytes, neutrophils, erythrocytes, platelets (fluorescence flow cytometry) – the reference analyser in our hospital.
HemoScreen™, PixCell™: haemoglobin, leukocytes, neutrophils, erythrocytes, platelets (Viscoelastic focusing and Artificial Intelligence Machine Vision Analysis)
Statistics: GraphPad Prism™ e MedCalc™ (Passing-Bablok regression, Spearman rank correlation coefficient)
The coefficients of variation (CV%) for the analytes tested on the HemoScreen were calculated from five replicates of samples.
Evaluation of daily internal quality control using matrices suitable for both devices.

Results: The samples were tested in both equipments. The CV(%) for HemoScreen™ were: WBC=3.4 (8,98-9,70), RBC=2,7 (4,1-4,3), HGB=2,8(12,52-13,25) e HCT=2,8(36,85-39,00), MCV=0,6 (89-90), PLT= 6,5 (254-301), NEU=7,5 (3,62-4,41) e LYM= 8,8 (1,70-2,05). The correlation coefficient (r) between the two equipment were WBC (r=0,99), RBC(r=0,99), HGB (r=0,99), HCT(r=0,98), MCV (r=0,84), PLT (r=0,91), NEU (r=0,98) e LYM (r=0,98).

Conclusion: The results were equally accurate on both devices and the differences are not clinically relevant.
HemoScreen™ is easy to use, requires no maintenance or specialized staff and delivers fast results. These features are very useful and are suitable to be used by doctors and nurses in primary care centers, home care services, and hospital care settings, as well as a backup device for hospital laboratories within the ULS.

P20

HEMOLYTIC ANEMIA (HA) AND MYELODYSPLASTIC SYNDROME (MDS): A CLINICAL CASE REPORT

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Introduction: Myelodysplastic Syndrome are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis, peripheral cytopenias, and cellular dysplasia. Some patients with MDS develop hemolysis, which is often of non-immune etiology, associated with ineffective intramedullary erythropoiesis, acquired hemoglobinopathies, and defects in erythrocyte membranes.
Laboratory markers suggestive of non-immune HA include increased lactate dehydrogenase (LDH) and indirect bilirubin, low or undetectable haptoglobin, elevated reticulocytes, erythrocyte fragments in the peripheral blood smear (PBS), and a negative Direct Antiglobulin Test (DAT).

Clinical case: An 80-year-old woman was referred to the Hematology clinic for bicytopenia, dizziness, and frequent haematomas. On physical examination, she was pale, with no palpable lymphadenopathy and no other significant findings. Laboratory results showed: Hb 6.8 g/dL, Leukocytes 4000/µL, PLT 97 x 10^3/µL, Total Bilirubin 2.2 mg/dL, Uric Acid 9.7 mg/dL, Haptoglobin undetectable, LDH 803 U/L, EPO 70 mU/mL, and a negative DAT. The PBS revealed erythroblasts and macroplatelets. The patient was hospitalized for further investigation of HA. Complementary diagnostic tests carried out during hospitalization included: a computed tomography scan of the chest, abdomen, and pelvis which showed a mildly enlarged spleen (13.7 cm); the echocardiogram revealed mild mitral insufficiency and atrial dilation; the JAK2 V617F mutation test was negative (myelofibrosis was hypothesised); the bone marrow biopsy showed hypercellular marrow with increased hematopoietic lineages, significant morphological abnormalities in megakaryocytes, and an excess of blasts (10-19%); the karyotype was 46XX(20), and the FISH did not show any abnormalities. Based on the clinical presentation and alterations detected in the tests, the diagnosis of MDS with excess blasts was established, and treatment with Azacitidine and Epoetin initiated.

Conclusion: This case highlights the importance of considering MDS as a potential cause of non-immune hemolysis, particularly in patients with concurrent cytopenias. Although hemolysis is not a typical presentation of MDS, it can significantly complicate the clinical picture and pose diagnostic challenges. In this case, bone marrow analysis was crucial for establishing the diagnosis, allowing for a more appropriate therapeutic approach.

P21

MYELODYSPLASTIC /MYELOPROLIFERATIVE NEOPLASM WITH RING SIDEROBLASTS, THROMBOCYTOSIS AND SF3B1 MUTATION: CHALLENGES IN DIAGNOSIS

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Introduction: Myelodysplastic /Myeloproliferative Neoplasm with Ring Sideroblasts and Thrombocytosis (MDS/MPN-RS-T) is a subtype of MDS/MPN characterized by persistent anemia, thrombocytosis (≥450 × 10^9/L), less than 1% blasts in peripheral blood, at least 15% ring sideroblasts, and fewer than 5% blasts in the bone marrow.
The SF3B1 mutation is present in 60–90% of cases, frequently associated with JAK2 V617F and, less commonly, with CALR or MPL mutations. These genetic alterations explain the proliferative aspects of the disease and influence prognosis. The disease may progress to myelofibrosis or acute myeloid leukemia. There is no specific treatment, and management is tailored to the clinical presentation of each patient.

Aim: To present a clinical case of MDS/MPN-RS-T.

Case Report: A 61-year-old female patient with a history of type 2 diabetes mellitus presented with headaches, dizziness, gait imbalance, unintentional weight loss of 20 kg over the past year, fatigue, and night sweats.
Laboratory findings revealed normocytic normochromic anemia (hemoglobin: 11 g/dL) and significant thrombocytosis (1,147,000/L). Peripheral blood smear showed anisopoikilocytosis, platelet anisocytosis, and giant platelets. Based on these findings, hydroxyurea was initiated (500 mg, 14 tablets/week). A bone marrow biopsy and aspirate were performed. The aspirate showed hypercellular bone marrow with an altered myeloid-to-erythroid ratio due to erythroid hyperplasia and signs of dyserythropoiesis in more than 10% of cells, with 50% ring sideroblasts confirmed by Prussian blue staining. Blasts and megakaryocytes accounted for 2% and 0.5% of total cellularity, respectively.
Molecular testing for BCR-ABL, JAK2, and CALR mutations was negative, while the SF3B1 mutation was positive. Laboratory findings were consistent with a diagnosis of MDS/MPN-RS-T with SF3B1 mutation. The patient remains under follow-up in the hematology clinic.

Conclusion: This clinical case illustrates the characteristics of MDS/MPN-RS-T, emphasizing the importance of morphological and molecular evaluation in the diagnosis and follow-up of this disease. Despite the cytotoxic effects of hydroxyurea on hematopoiesis, which may interfere with morphological assessment, the presence of ring sideroblasts, even in the context of myeloproliferative features, and the detection of SF3B1 mutation strongly supported the diagnosis. These findings were crucial for disease classification and prognostic evaluation.

P22

UNESPECTED S “LIKE” RARE HEMOGLOBIN - CLINICAL CASE REPORT

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Introduction: Non-sickle hemoglobin (Hb) variants, which can elute in the HbS window in high performance liquid chromatography, can pose diagnostic challenges in certain equipment, especially in HbS prevalent geographies, such as the Alentejo region. Thus, we believe it is important to identify and distinguish the silent variants from those with clinical significance.

Case report: Here we describe the case of a 55-year-old man, with no known foreign ancestry, who was referred to our laboratory for diabetes monitoring. His complete blood count was normal and there were no significant findings in the blood smear.

The chromatogram showed an abnormal Hb peak (HbX) in the Hb S window in Hb Next Menarini (21%) and an extra peak in 8180T Arkray (20.4%) with a higher retention time than Hb A2. Alkaline electrophoresis and isoelectric focusing on polyacrylamide gel revealed a band in the Hb S area.
Hb S solubility test was also performed, which was negative.
In order to better characterise this hemoglobin, a molecular study was carried out at a reference laboratory (INSA Dr. Ricardo Jorge), where mutation analysis of the alpha globin gene was performed by DNA sequencing. The results showed heterozygosity for the c193G>A p.(Asp65Asn) condition. According to the literature, the HBA2 mutation leads to Hb G-Waimanalo, which, to our knowledge, is a very rare variant in Portugal. Hb G-Waimanalo was first observed in members of a Filipino family and often results in mild microcytic hypochromic anaemia or normal erythrograms when present in heterozygosity, as in the case described.

Discussion: Most screening for haemoglobinopathies is based on systematic analysis of chromatograms in individuals who are unaware of their condition.
This case therefore highlights the importance of screening for hemoglobin variants, as it is essential for better characterisation of these genetic variations in order to appropriate patient's clinical management, controlling the risk of severe phenotypes to the carriers' offspring and eventual genetic counselling.
Further family studies could be useful to understand whether it is a de novo heterozygous variant.

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P23

HB C TRAIT IN A PREGNANT WOMAN - CASE STUDY

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Introduction: Hemoglobin C (Hb C) is the third most common hemoglobin (Hb) variant in the world with genetic origins in Africa, southern Europe, and Thailand. Hb C is a structural variant caused by a point mutation in the hemoglobin β locus (HBB) resulting in the substitution of glutamic acid with lysine at position 7 of the β-globin chain (p.Glu7Lys; c.19G>A). Heterozygosity for Hb C (Hb C trait), is a benign carrier state that does not require clinical intervention.
However, genetic counseling and testing for hemoglobinopathies should be offered to individuals with this trait to assess the risk for affected offspring.(1, 2)

Aim: This report concerns a 19-year-old female patient referred to our hospital for evaluation of pregnancy anemia.

Methods: Complete Blood Count (CBC), microscopic observation of peripheral blood smear (PBS), measurement of Hb fractions using High Performance Liquid Chromathography (HPLC) technique, DNA extraction and amplification by Polimerase Chain Reaction followed by Sanger sequencing of the HBB gene.

Results and discussion: CBC and microscopic observation of the PBS revealed marked hypochromia and anisocytosis with a predominance of target cells, microcytic and some irregular erythrocytes. HPLC revealed an increased Hb A2 (3,4%), a decreased Hb A0 (56,6%) and the presence of an abnormal peak (31,7%), possibly corresponding to a Hb variant. The genetic study confirmed the presence of the heterozygous point mutation c.19G>A p.(Glu7Lys) corresponding to the cDNA genotype HBB: c.[19G>A][19=] compatible with the Hb C trait.
The born child was screened at 5 months of age. CBC and microscopic observation of the PBS revealed moderate hypochromia and anisopoikilocytosis, with the presence of microcytic erythrocytes, some helmet cells and rare target cells. HPLC revealed 12,9% HbF, 3,1% Hb A2, 48,5% Hb A0 and the presence of 31% HbC.

Conclusion: Both the patient and her child have Hb C trait, a benign carrier state that does not require clinical intervention. Partner testing and future preconception counseling are highly recommended to assess the risk of this couple's offspring for Hb C disease or other possible inherited hemoglobinopathies.(2) Information about the condition and future reproductive options should be discussed.(3) Screening for hemoglobinopathies is increasingly important due to the expanding ethnic and geographic diversity of hemoglobinopathy genotypes worldwide.(3)

P24

LET´S PUT A RING ON IT.

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Introduction: Malaria is a leading cause of death worldwide, and early diagnosis is essential for successful treatment. According to the World Health Organization, microscopic examination remains the gold-standard for diagnosis.
However, we should be aware of certain laboratory alerts for faster and more targeted diagnosis in the face of clinical suspicion.

Case Report: A 25-year-old black woman from Luanda, presented to the emergency department of a tertiary hospital 24 hours after her return from Angola, with fever of 5 days' duration, asthenia, dizziness, night sweats and haematuria. On observation, she was hypotensive (blood pressure 80/40 mmHg), icteric and febrile (temperature 39ºC).
Blood tests showed pancytopenia (leukocytes 2.43*10^9/L, haemoglobin 8.7g/dL, platelets 41.0*10^9/L), lactate dehydrogenase 651UI/L, total bilirubin 9.3mg/dL (indirect bilirubin 3.65mg/dL), C-reactive protein 28.14mg/dL and procalcitonin 3.04mg/dL. The reticulocyte analysis (RET) scattergram (Sysmex XN-10) showed an abnormal population at the site of immature reticulocytes.
In view of these findings, the Clinical Pathologist carried out a peripheral blood smear, which revealed a count of 340 parasitised erythrocytes out of 1000 total erythrocytes (34%), of which the presence of multiparasitised erythrocytes stood out, some showing 3-4 rings, suggestive of Plasmodium falciparum infection, a species confirmed by a rapid antigen test.
In the face of severe malaria, she started intravenous artesunate therapy, with rapid clinical and laboratory improvement and a rapid drop in parasitaemia.

Discussion: The aim of this case is to highlight the importance of clinical suspicion, which in the face of an abnormal population in the leukocyte differential scattergram or an abnormal reticulocyte cluster in the RET scattergram may indicate the presence of parasitised erythrocytes, as demonstrated in multicentre studies. In the case of the RET scattergram, the distribution of cell populations may also indicate the presence of uni- or multi-parasitised erythrocytes.
This allowed the patient to receive appropriate and prompt treatment, resulting in a good clinical and analytical evolution in a disease with a significant mortality rate.

P25

ACUTE KIDNEY INJURY AS THE INITIAL MANIFESTATION OF MULTIPLE MYELOMA - CASE REPORT

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Case Report: An 84-year-old male patient presented to the emergency department with anorexia and generalized muscle weakness. His medical history includes treated oropharyngeal cancer (13 years ago), acute myocardial infarction, heart failure with preserved ejection fraction, gout and hypothyroidism. On physical examination, he appeared dehydrated with dry skin and mucous membranes.
Initial laboratory evaluation showed leukocytosis, normocytic/normochromic anemia, thrombocytosis and the presence of plasmocytes and rouleaux formation. Associated with altered renal function, with urea of 143 mg/dL, creatinine of 6.18 mg/dL, hyperkalemia (7.1 mEq/L), hyperuricemia (15.5 mg/dL) and hypercalcemia (10.5 mg/dL). Additionally, there was cholestasis and elevated gamma-GT (195 U/L).
To continue the investigation, the protein electrophoresis showed a monoclonal peak in the gamma region (IgA/Kappa (K)) in both serum and urine. This was confirmed by elevated IgA (2180 mg/dL) with reduced IgG and IgM and increased K with undetectable Lambda.
The bone marrow biopsy revealed plasmacytosis (47%). Other markers such as erythrocyte sedimentation rate and beta2-microglobulin were elevated. The CT scan did not reveal any lytic lesions.
With the diagnosis of IgA/K Multiple Myeloma (MM) confirmed on the 11th day of hospitalization, treatment with dexamethasone (20 mg/day for 5 days) was initiated.
Despite the initial management of hyperkalemia and the introduction of dexamethasone, the patient maintained significant renal injury (creatinine 4.66 mg/dL). He progressed to cardiorespiratory arrest and passed away on the 16th day of hospitalization.

Discussion: This case highlights the importance of considering monoclonal gammopathies in elderly patients with acute kidney injury of unclear origin. The presence of plasmocytes and rouleaux in the blood smear was a crucial diagnostic clue.
Although less common than IgG, IgA/K monoclonal gammopathy is often linked to early renal involvement. The absence of lytic bone lesions on the CT emphasizes the heterogeneity of multiple myeloma.
This case underscores the need for timely recognition and management of MM.
Early diagnosis can improve treatment outcomes and survival, even in the presence of severe complications like acute kidney injury.

P26

ACUTE PROMYELOCYTIC LEUKEMIA: A CASE REPORT

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Background: Acute Promyelocytic Leukemia (APL) is a rare and distinct subtype of Acute Myeloid Leukemia (AML), typically associated with the PML-RARA fusion gene, resulting from a reciprocal translocation between chromosomes 15q22 and 17q21.

This genetic alteration disrupts normal promyelocyte differentiation, leading to the accumulation of immature cells in the bone marrow (BM) and/or peripheral blood. Standard treatment involves all-trans retinoic acid (ATRA) to induce differentiation into mature granulocytes, in combination with arsenic trioxide (ATO) or
chemotherapy to achieve complete remission.

Case Report: A 65-year-old woman was admitted to the emergency department on January 27, 2025, after a ground-level fall with head trauma and loss of consciousness. Following the event, she experienced urinary incontinence and vomiting.
She reported a three-week history of fatigue and easy bruising. On admission, her blood pressure was 96/64 mmHg, and she continued to vomit. A cranial CT scan showed no acute intracranial lesions.
Laboratory findings revealed severe anemia (hemoglobin: 5.1 g/dL), thrombocytopenia ($33x10^3/\mu L$), neutropenia ($0.89x10^3/\mu L$), prolonged prothrombin time (18.7 sec), low fibrinogen (66 mg/dL), and an elevated INR (1.69).
A peripheral blood smear showed 4% blasts with Auerrods and 24% promyelocytes with coarse granulation, raising suspicion for APL. ATRA therapy was initiated immediately.
Bone marrow aspiration revealed a moderately hypocellular BM with 66% atypical, hypergranular promyelocytes containing Auer rods. Cytogenetic analysis confirmed the presence of the t(15;17) translocation, and genetic studies identified the PML-RARA rearrangement along with an FLT3-ITD mutation. Consequently, ATO was added to the ongoing ATRA treatment, leading to a favorable clinical outcome.

Conclusion: APL is a hematological emergency due to the high risk of severe and potentially fatal coagulopathy, often associated with disseminated intravascular coagulation with hyperfibrinolysis. This case highlights the importance of initiating ATRA therapy as early as possible, ideally before definitive confirmation of the diagnosis, to improve prognosis and reduce potential complications in these patients.

P27

UNUSUAL ERYTHROCYTE AGGREGATION IN A 73-YEAR-OLD FEMALE: A CASE SUGGESTIVE OF COLD AGGLUTININS

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Introduction: A 73-year-old female routine blood tests, revealing significant erythrocyte aggregation at room temperature (RT). Due to this finding, a complete blood count (CBC) was performed after heating (AH) the sample to 37°C,30-60 minutes. This case raises the suspicion of cold agglutinins and highlights the importance of recognizing and managing temperature-sensitive hematologic abnormalities. Cold Agglutinin Disease (CAD) is a rare autoimmune hemolytic anemia (AIHA) where cold-reactive autoantibodies bind to red blood cells (RBC), causing erythrocyte aggregation and hemolysis. This condition can be primary (idiopathic) or secondary to infections (e.g., Mycoplasma pneumoniae, EBV) or lymphoproliferative disorders. CAD can interfere with automated CBC measurements, requiring temperature corrections for accurate medical diagnosis.

Patient Details: 73-year-old female; Laboratory Findings: CBC abnormalities: anisocytosis, macrocytosis, polychromatophilic erythrocytes; smear images confirm erythrocyte agglutination; significant correction of RBC count (RT=1.47x109/L); AH=3.62(x109/L); Mean Cell Hemoglobin Concentration(MCHC) (RT=80,6g/dL; AH=35,7g/dL);Mean Corpuscular Hemoglobin (MCH) (RT=90.5pg;AH=36.7pg);Mean Corpuscular Volume (MCV) (RT=113.1fL;AH=103.0fL); platelet count: 340(x109/L). Hematological parameters were performed using the Sysmex XN-10 system.

Discussion and Clinical Relevance: Erythrocyte aggregation at RT can lead to inaccurate CBC, MCHC, MCH and MCV readings, emphasizing the importance of sample heating in suspected cases; CAD can cause chronic hemolytic anemia and complications in cold environments.
Other causes of RBC aggregation must be considered: “rouleaux formation”:seen in multiple myeloma or chronic inflammatory conditions, but typically doesn´t solve with heating; cryoglobulinemia: presence of cryoglobulins can cause hemolysis and cold-induced circulatory issues. Further investigations should include: direct antiglobulin test (DAT) with complement (C3d) evaluation; cold agglutinin titer; hemolysis markers; workup infections or malignancies.

Conclusion: This case serves as an important reminder of the diagnostic challenges of erythrocyte aggregation at RT. Recognizing cold agglutinins can prevent CBC misinterpretation and guides further testing. The results were reported with the following text:
"This determination was provided after heating the blood sample to 37ºC because erythrocyte aggregation was detected at room temperature.".

P28

SICKLE CELL ANEMIA: A CASE REPORT

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Introduction: Sickle cell anemia is a rare hemoglobinopathy caused by a mutation in the β-globin gene, leading to hemoglobin S (HbS) production and chronic hemolytic anemia. Patients experience recurrent vaso-occlusive crises (VOCs) that impact quality of life. Though most prevalent in African populations, global migration has expanded its reach. In Portugal, prevalence is uncertain, but an estimated 1 in 2,500 newborns is affected, underscoring the need for increased awareness and specialized care.

Case report: A 23-year-old woman with a known history of sickle cell anemia presented to the Emergency Department on September 10, 2024, with fatigue and severe bilateral knee pain (8/10). Originally from Angola, she had prior VOCs requiring one to two transfusions annually.
On examination, she had pale mucous membranes and tenderness in her calves and knees. Laboratory findings included:

• Anemia: Hb 9.6 g/dL, microcytosis (MCV 68 fL), hypochromia (MCH 22 pg)
• Leukocytosis: 21.65 × 10^3 cells/µL, with neutrophilia (17.71 × 10^3 cells/µL)
• Hemolysis & inflammation markers: Indirect bilirubin 3.18 mg/dL, LDH 590 U/L, CRP 102 mg/L

Morphine was started but provided insufficient relief, leading to hospitalization in the Hematology Department. Hydroxyurea was initiated, and pain improved by day two. HbS quantification (HPLC) revealed: HbA2 6%, HbF 1.1%, HbS 81.9%.
On day three, she developed another VOC with severe pain and Hb drop to 6.5 g/dL, requiring urgent exchange transfusion. After stabilization and symptom resolution, she was discharged with outpatient follow-up in the Red Blood Cell Pathology clinic.

Conclusion: This case highlights the growing need for early diagnosis, specialized management, and structured care pathways for sickle cell anemia in Portugal.
As migration reshapes disease epidemiology, ensuring access to hydroxyurea, transfusions, and comprehensive care is essential to improve patient outcomes and quality of life.

P29

A SIMPLE ANEMIA OR MULTIPLE MYELOMA? A CASE REPORT

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Introduction: Multiple Myeloma is a hematologic cancer defined by malignant clonal proliferation of plasma cells in the bone marrow, resulting in increased production of immunoglobulins, secretion of paraprotein in serum and/or excretion of pathologic free light chains in urine. These conditions contribute to organ damage, characterized by hypercalcemia, renal dysfunction, anemia, and osteolytic lesions. It is the second most prevalent hematologic malignancy, with 188 000 diagnosed cases and 121 000 deaths estimated worldwide in 2022.
It has a higher incidence in individuals of African descent compared to Caucasians, as well as in males compared to females. The average age at diagnosis is 66 years, highlighting the importance of early diagnosis and treatment of this poor prognosis entity.

Case report: A 61-year-old male patient presented to the emergency department with a diagnosis of anemia, for which he is currently being followed up in primary healthcare. Upon admission, the patient was asymptomatic, reporting an abrupt decrease in serum hemoglobin concentration detected in previous blood tests. Following an analytical investigation with a complete blood count, hemoglobin was found to be 6.9 g/dl and two units of red blood cell concentrate were transfused. Additionally, macrocytic anemia and neutropenia were identified, prompting the performance of a peripheral blood smear, which revealed 17.5% plasma cells and marked rouleaux formation, both findings suggestive of monoclonal gammopathy. In light of the suspected diagnosis, serum protein electrophoresis, free serum total light-chain assay, creatinine and total protein levels were performed.

The suspected features of Multiple Myeloma were confirmed, the patient was referred to a Clinical Hematology consultation at a tertiary hospital and discharged from the emergency department with a referral letter to the primary care physician.

Discussion: The study conducted at our hospital revealed hyperproteinemia and a monoclonal peak in the gamma region, corresponding to an immunoglobulin G with kappa light chains. Therefore, the prompt diagnostic approach in the emergency setting allowed for timely and thoughtful referral of the patient to a tertiary hospital, where he is currently receiving treatment.

P30

UNMASKING PSEUDOMONOCYTOSIS: A CASE REPORT OF LYMPHOPROLIFERATIVE DISEASE

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Case Report: A 70-year-old woman with chronic hepatitis B underwent a routine blood test. The automated CBC revealed hemoglobin 14.5 mg/dL, leukocytes $5.8x10^3$/µL, neutrophils $2.5x10^3$/µL (43.8%), lymphocytes $1.4x10^3$/µL (24.3%), monocytes $1.8x10^3$/µL (30.3%), and platelets $195x10^3$/µL. The scatter plot showed well-defined leukocyte populations, with monocytosis as the only flag. A Peripheral Blood Smear (PBS) revealed atypical lymphoid cells (twice the size of small lymphocytes, fine chromatin, and exuberant nucleolus), requiring a count correction, which was: neutrophils $2.7x10^3$/µL (46%), monocytes $0.2x10^3$/µL (4%), lymphocytes $1.2x10^3$/µL (20%), and atypical lymphoid cells $1.7x10^3$/µL (29%). Immunophenotype revealed lymphocytes expressing CD5+, CD19+, lambda light chain restriction, CD20+, CD25+, and CD79b+, which suggests B-cell lymphoproliferative disease. The patient was referred to a clinical hematology consultation. In subsequent CBC, pseudomonocytosis persisted.

Discussion: The presence of scatter plots with well-defined leukocyte populations may falsely suggest the absence of cytological alterations. However, well-established criteria for observing PBS prevent the omission of critical morphological cell observations. As such, this case is a significant paradigm for applying the rules and criteria for observing PBS, considering the technical limitations of each autoanalyzer.

Conclusion: We, therefore, conclude that the rules for requesting and observing PBS are fundamental for an accurate diagnosis of hematological pathology.

P31

CUTANEOUS LEISHMANIASIS: A CLINICAL CASE REPORT WITH LABORATORY DIAGNOSTIC INSIGHTS

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Introduction: Leishmaniasis is a vector-borne disease caused by Leishmania protozoa, transmitted through sandfly bites. It primarily manifests as visceral or cutaneous leishmaniasis (CL), with the clinical presentation influenced by the specific Leishmania species involved. Accurate species identification, along with geographic location and the host's immune response, is crucial for effective management. We report a case of CL in a Portuguese male presenting with a hallux lesion.

Case description: A 70-year-old caucasian male, immunocompromised due to a history of lymphoma and insulin-treated diabetes mellitus, presents a 15-year recurring lesion on the right hallux, characterized by cycles of healing and relapse. Over the past 6 months, the lesion worsened significantly, without any prior local trauma. It had a fetid odor but was asymptomatic and painless. On physical examination, a 5×5 cm verrucous tumor circumferentially involved the hallux, with focal erythematous and brownish papillomatous areas. No additional limb lesions or palpable lymphadenopathy were noted.
Histopathological analysis showed marked epidermal hyperplasia with a verrucous and ulcerated appearance, heavily infiltrated by histiocytes containing multiple parasitic structures consistent with amastigotes. PCR and sequencing confirmed L. donovani/infantum/chagasi complex.
Blood serology for Leishmania was positive by Chemiluminescence immunoassay IgG/IgM (Index 4.8, cut-off <0.9), Indirect immunofluorescence assay (1:320) and Western blot IgG, while bone marrow biopsy showed no evidence of amastigotes.
He visited Tunisia and Morocco 15–20 years ago and Israel 10 years ago, but never the Americas or elsewhere. He had regular contact with animals, including 2 pet dogs. He was hospitalized 30 years ago for 1 month of intravenous therapy for suspected kala-azar, which was apparently not confirmed.
The patient was diagnosed with chronic CL, without mucosal or visceral involvement, and started on liposomal amphotericin B.

Discussion: This case highlights the diagnostic challenges and clinical implications of CL. Laboratory findings played a crucial role in confirming the diagnosis, as the chronic and recurrent nature of the lesion made clinical suspicion alone insufficient.
The integration of histology, molecular diagnostics and serology was fundamental in establishing the diagnosis, emphasizing the need for a comprehensive laboratory approach when evaluating persistent cutaneous lesions.

P32

CAMPYLOBACTER INFECTION IN A NEWBORD: CASE REPORT

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Introduction: Campylobacter jejuni (C.jejuni) is a common cause of gastroenteritis in children and adults, but its occurrence in newborns is rare and can lead to serious systemic manifestations, including bacteremia. We report a case of neonatal bacteremia caused by C.jejuni, highlighting the importance of early recognition and an appropriate therapeutic approach.

Case report: A previously healthy newborn, was admitted to the emergency room with a fever of 39°C, liquid diarrhea, food refusal and colic. Laboratory analysis showed anemia, low white blood cell count, high C reactive protein levels and high procalcitonin. A lumbar puncture was performed, the cytological examination of which showed some cellularity, absence of glucose consumption and slight proteinorrhaquia. The molecular panel for cerebrospinal fluid infectious agents was negative. Empirically, ampicillin and cefotaxime were started.
One blood culture was obtained and collection of feces for bacteriological examination. The virological examination of the feces was negative. After 48h, characteristic colonies grew in selective medium and microaerophilic environment. Blood culture were positive after 102,8 hours. The Gram stain showed Gram-negative, comma-shaped rods.
Identification in matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) revealed to be C.jejuni. The agent was susceptible to azithromycin and ciprofloxacin resistant. The antibiotic was adjusted and the newborn completed 5 days of azithromycin. At the end of treatment, inflammatory markers were reduced, and the patient was discharged with follow-up in neonatology.

Discussion: C.jejuni is a common cause of diarrhea in children and adults, but its involvement in neonatal bacteremia is uncommon.
Transmission can occur through contact with contaminated food, animals or maternal-fetal routes. In newborns, the infection may present with fever and nonspecific gastrointestinal symptoms, which may progress to sepsis. This case highlights the need to consider C. jejuni as an etiological agent in neonates with fever and gastrointestinal symptoms, especially when we have systemic involvement. Stool culture and blood culture are essential for diagnosis and treatment should be guided by antimicrobial susceptibility tests in these more serious cases.

P33

WHAT DO WE BRING FROM TRAVELING ABROAD? A CLINICAL CASE OF ENTAMOEBA HISTOLYTICA

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Introduction: Different protozoan species of Entamoeba can colonize humans, but only a few are responsible for causing disease. Entamoeba histolytica is a pathogenic amoeba, associated with intestinal and extraintestinal infections, causing dysentery and liver damage. It's found in freshwater contaminated with human feces and is more frequent in developing countries 1. The risk groups in developed countries are travelers, recent immigrants, immunocompromised, men who have sex with men and institutionalized people1. This clinical case reports an Entamoeba histolytica infection.

Case description: A 26-year-old male came to the emergency service with fever, nausea, vomiting, diarrhea, and holocranial headaches with significant retro-orbital pain without skin alterations or other complaints.
The patient presents an asthma history, controlled with inhalers, and is unaware of drug allergies. He spent around 2 months in Ivory Coast for work purposes.
On examination, he presented shivering with rising temperatures (37.5oC) and mild polypnea. A boold culture was negative. There were no biochemical alterations, except a C-reactive protein (CRP) of 1.04 mg/dL (<0.5 mg/dL).

The complete blood count only shows alterations on leucogram: slight leukocytosis of 12.8 x109/L (3.9-10.2 x109/L), with neutrophilia of 12.04 x109/L (1.50-7.70 x109/L), and lymphocytopenia of 0.35 x109/L (1.10- 4.50 x109/L). Seasonal viral disease, malaria, dengue, tick-borne diseases, and other common infections were excluded. Paracetamol was administered and the patient was discharged with Doxycycline 100mg 2 id. The patient returned for evaluation. The leucogram was normalized, the CRP increased to 4.87 mg/dL, and antibodies to Amebiasis were detected by indirect hemagglutination. Charcot-Leyden crystals were observed in the patient's feces. X-ray and abdominal ultrasound were normal. This case was classified as a presentation of extra-intestinal amebiasis.

Discussion: Migration, as temporary work or tourism, especially to developing countries, continues to rise, so diseases commonly found in these regions are becoming more prevalent in the developed world. This clinical emphasizes the importance of following these patients and their laboratory findings, allowing the detection of less common pathologies and adoption of appropriate strategies to optimize the diagnosis and treatment.

P34

MYCOBACTERIA SPECIES IDENTIFICATION: IMMUNOBLOT VS LIQUIDARRAY - A COMPARATIVE STUDY

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Mycobacteria are aerobic, non-motile organisms that test positive with acid-fast alcohol staining. Nontuberculous mycobacteria (NTM) are species distinct from those within the Mycobacterium tuberculosis complex (MTBC). To date, more than 140 NTM species have been identified.
The identification of NTM species using Immunoblot is truly subjective, as the results depend on the interpretation of a band pattern. In these patterns, certain bands must be disregarded due to their weak intensity compared to the control band. In our laboratory, we use a Genotype® kit from Bruker. We aim to compare this method with NTM species identification using the LiquidArray system and fluorotype kit in the FluoroCycler®XT by Bruker.
After amplification, the LiquidArray provides an identification of the mycobacterial species, or confirms their absence, with the aid of artificial intelligence. The FluoroCycler®XT software compares our amplification curves with its database of amplification curves from 32 NTM and MTBC species.

For this study, we analysed 100 positive culture samples using both methods. Concordant results were obtained in all cases, except where the genotype identified the M. fortuitum group and M. intracellulare. This discrepancy arises because the genotype for the M. fortuitum group includes four species (M. fortuitum, M. peregrinum, M. alvei, and M. septicum) that share the same band pattern, while the fluorotype kit only has primers to identify two of these species, providing results for M. fortuitum and M. peregrinum. For M. alvei and M. septicum, the fluorotype gives a result of M. spp.
Regarding M. intracellulare, the bands in the genotype could also indicate M. marseillense and M. chimaera (both from the M. avium complex), as they present the same band pattern in the immunoblot.

The fluorotype can distinguish between these species, identifying M. intracellulare and M. chimaera, but provides a result of M. spp. for M. marseillense, as this kit does not have specific primers for these species.

We conclude that the LiquidArray method for identifying mycobacterial species offers several advantages over immunoblotting. It allows identification in half the time (2 hours), is more accurate, less subjective, and does not require human visual analysis of band patterns obtained with a non-automated method.

P35

EVALUATION OF KINGELLA KINGAE DETECTION BY REAL-TIME PCR IN PEDIATRIC SAMPLES

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Introduction: Kingella kingae (Kk) is recognized as an important cause of septic arthritis infections (SAI) in young children.
Although considered to be benign, with a mild to moderate clinical presentation and a favorable prognosis after antibiotic treatment, it requires a prompt diagnosis and treatment. Establishing the diagnosis is particularly difficult since the specimens from de infected site are not always available. Several studies demonstrated that when Kk is identified, with specific real-time PCR on an oropharyngeal samples (OFS) it may provide a strong evidence that Kk is responsible of SAI. Therefore, our goal was to evaluate the results of Kk detection in suspected pediatric samples.

Results: From 2023-2024, 172 samples (oropharyngeal and synovial fluid) were evaluated by Kk specific real-time PCR. 109 samples were from children ≤ 2 years old, 44 from ≤5 years old, and 19 from ≤16 years old. 162 were OFS: 63 were positive (38,89%), and 99 were negative (61.11%). 21 were synovial fluid samples (SFS): 13 positive(61,90 %), 8 negative (38.10%). In 10 children OFS and SFS were simultaneously collected: Kk PCR was performed with an agreement of 90% (6/6 positive; 3/3 negative; 1 OFS positive/SFS negative). All the positive Kk were found in the ≤ 2 (70%) and ≤5 (5%) age groups.

Discussion: A positive result of Kk in OFS in children under 5 with SAI provides strong evidence that Kk is the etiologic agent of infection. However, in the positive cases it is not possible to exclude colonization. To clarify these cases it is important to correlate the clinical complains and to collect a SFS. Our results showed that a negative results rule out Kk as the causative pathogen.

P36

AUTOMATED ALINITY M PLATFORM: PERFORMANCE ASSESSMENT OF THE STI ASSAY

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Sexually transmitted infections (STIs) are a global public health problem with more than 1 million people acquiring a STI every day. Accurate diagnostic of STIs is essential for timely administration of appropriate treatment and contact surveillance to avoid the spread of the disease.

Several STI diagnostic systems are available and differ regarding their performance, number of parameters outputs, turnaround time and cost. Real time PCR Alinity m STI assay runs on the fully automated, continuous, and random-access molecular platform, which not only improve diagnosis quickness but also flexibility in the management of laboratory testing.

The aim of this work was the evaluation of the performance of Alinity m STI assay to detect Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Trichomonas vaginalis (TV), and Mycoplasma genitalium (MG) in a single reaction.

A total of 98 clinical and 10 external quality control samples were tested in parallel with both assays: Alinity m STI and Real Time PCR multiplex manual assay.
Overall, the Alinity m assay showed a sensitivity of 100% and a specificity of 96.6%. When stratified the agreement for each parameter the results were as follows:
CT: the positive (PPA) and negative (NPA) agreement were 100% and 98.9% respectively; NG: PPA of 100% and NPA of 98.9%; MG: PPA and NPA were 100% and for TV, NPA was 100% (no positive results obtained).
In total, 4 false positives were detected in male urine samples (3/CT and 1/NG). However, all of them showed low copy number of the target DNA, close of the detection threshold of the test.
Additional, external quality control samples showed 100% concordance with the score reported and all panel members were detected and accurately identified individually or in the presence of the other pathogens.
This system allows process standardization and the ability to perform any test in any time since this platform allows the continuous processing of different samples in parallel, combined with the simultaneous examination of several microorganisms. In addition, operator error is reduced because the three stages of real-time PCR are performed within the platform.

In conclusion, this system provides a rapid response to the clinician in terms of identifying the four main microorganisms responsible for the STIs and is an excellent tool for controlling the spread of infection between partners.

P37

MULTIPLEX PCR IN CHILDREN'S ACUTE RESPIRATORY TRACT INFECTIONS

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Introduction: Acute respiratory infections (ARTI) in children are one of the main causes of healthcare seeking, especially during the autumn and winter months. Most of the ARTI are of viral origin and self-limited. However, ARTI may also have bacterial origin, in which case a specific treatment is required. Since viral infection is often difficult to distinguish from bacterial infection the use of a syndromic multiplex panel to detect viruses and bacteria in respiratory secretions is potentially beneficial. Our aim was to analysis the pathogens identified in respiratory secretions from ARTI, in children during the 2024 year.

Methods: Respiratory secretions were collected by nasopharyngeal swabs (NPS).

Swabs were tested using the FilmArray system (FilmArray® Respiratory Panel, Idaho Technology) that detects by real-time PCR, 22 targets: Adenovirus (ADV), Coronavirus 229E, HKU1, NL63, and OC43, SARS-CoV-2, Metapneumovirus, Influenza A, Influenza B (IB), Parainfluenza Virus 1-4, Rhinovirus/Enterovirus (RHV/EV), Respiratory Syncytial Virus (RSV), Bordetella pertussis (Bp) and parapertussis, Chlamydia pneumoniae (Chp) and Mycoplasma pneumoniae (Myp).

Results: 4144 NPS were analyzed between January and December 2024. 86% of the tests were requested by the Emergency Department. Respiratory pathogens were detected in 85.3 % samples. 49.7% of the positive samples were from children under 24 months. RHV/EV (in 49,2% samples), ADV (in 21.0% samples), IB (7.8% samples) and RSV (in 7.98% samples) were the most frequently detected viruses. An outbreak of Myp (14.5% of positive samples) particularly affected children over 12 months. Chp and Bp, were detected in 3.1% and 2.2% of the samples, most of them from children over 2 years old, respectively.

Conclusions: The use of a syndromic panel allows the rapid identification of pathogens, the implementation of adequate therapy, avoiding the unnecessary use of antibiotics and the identification of outbreaks. The test allowed the detection of Myp reemergence in the older children. ARTI associated to Myp are usually cyclical, with the last peak recorded in 2019-2020. From then until the second half of 2023, Myp remained almost indetectable. The reasons for this new outbreak are not clear but may be related to the atypical characteristics of this agent.

P38

EPIDEMIOLOGY OF LOWER RESPIRATORY INFECTIONS IN A TERTIARY HOSPITAL: ANALYSIS OF THE FIRST HALF OF 2024

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Introduction: Lower respiratory infections (LRIs) are a major cause of hospitalization and morbidity, especially among vulnerable populations. Understanding their epidemiology is essential for optimizing prevention, diagnosis and treatment strategies.
This study analyzed the epidemiology of LRIs in a tertiary hospital during the first half of 2024, focusing on case distribution, the principal etiological agents, and the laboratory methods employed.

Case Description: A total of 1276 cases diagnosed between January and June 2024 in patients over 18 years old were analyzed. These cases were categorized into four groups: inpatients, outpatients and day hospital attendees, intensive care medicine patients, and emergency department cases. Biological samples, including sputum, aspirates, and bronchoalveolar lavage, were processed using conventional microbiological methods (culture and Gram staining), with multiplex PCR employed in select cases for rapid etiological identification. Most cases were observed among inpatients (52.4%), followed by those in intensive care (23.7%). The predominant pathogens isolated were Streptococcus pneumoniae (30%), Staphylococcus aureus (25%), and Haemophilus influenzae (20%). Notably, the use of multiplex PCR reduced the time to obtain results dramatically, from 48 hours with traditional culture methods to just 45 minutes.

Discussion: The high prevalence of hospital-acquired LRIs highlights the need for effective preventive measures, such as strict hand hygiene and judicious antibiotic use. Furthermore, the adoption of rapid and sensitive diagnostic techniques like multiplex PCR can enhance early therapeutic intervention, leading to improved clinical outcomes and potentially shorter hospital stays. Continuous monitoring of epidemiological trends, including antimicrobial resistance profiles, is essential to refine and optimize control and prevention strategies.

P39

STRONGYLOIDIASIS: A NEGLECTED PARASITIC DISEASE

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Introduction: Strongyloidiasis is a helminth infection caused by Strongyloides stercoralis. Exposure to environments contaminated with feces is the main risk factor for acquisition, and the autoinfective life cycle can result in decades-long chronic infection that, if untreated, can persist in populations long after improvements in sanitation.
Strongyloides stercoralis is endemic globally, predominately in the South-East Asia, Africa, Western Pacific regions and in South and Central America.
Strongyloidiasis has a wide range of clinical presentations, including subclinical disease, symptomatic disease (often with diarrhoea and abdominal pain) and a rare but deadly complication of hyperinfection with disseminated disease.

Case Report: A 52-year-old woman presented to emergency department with a three-day history of vomiting and epigastric pain, with maintained bowel movements. She was afebrile and her full blood count was unremarkable (4,7 x 109 leukocytes and 0,13 x 109 eosinophils).
She is originally from Guinea and has been residing in Portugal since 1990. In ambulatory, one month before, she was diagnosed with duodenal stenosis of unknown etiology.
She was admited to the surgical unit, where a colonoscopy was performed and a biopsy was collected. The biopsy revealed duodenitis with Strongyloides stercoralis infection.
On D3 she was transferred to infectious disease department. Three consecutive stool samples were sent for parasitological examination, and Strongyloides stercoralis larvae were identified in all samples.
The patient completed 14 days of treatment with ivermectin.
HTLV-I IgG and Strongyloides stercoralis IgG tests were sent to an external laboratory, and both were reactive.
On D30 the patient was discharged with clinical improvement.

Conclusion: Hyperinfection/disseminated disease is associated with specific types of immunosuppression, such as that induced by HTLV-1.
Eosinophils play a prominent role in the innate immune response to Strongyloides stercoralis. Eosinophilia is frequently observed in people with chronic infection.
However, we didn´t observe this in the present case, because HTLV-1 decreases the Th2 response and reduces the activation of eosinophils and the production of IgE and cytokines (IL-4, IL-5), essential for immunity to helminth infections.
In an increasingly globalized world, this case highlights that parasitic diseases cannot be neglected.

P40

ANAEROBIC BLOODSTREAM INFECTIONS AT A TERTIARY HOSPITAL: PREVALENCE AND MICROBIAL PROFILE

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Introduction: Anaerobic bloodstream infections (BSIs) are less common than aerobic infections; However, they remain a significant cause of sepsis, especially in patients with risk factors such as recent abdominal surgery, trauma, or immunosuppression. Diagnosing these infections presents challenges that require clinical suspicion and specialized microbiological techniques. Understanding their epidemiology and management is essential for improving hospital treatment strategies.

Description of Casuistry: We conducted a retrospective observational study analyzing all anaerobic strains isolated from positive blood cultures between 2020 and 2024. The study aimed to assess the prevalence, microbial profile, and epidemiological significance of anaerobic bloodstream infections (BSIs) in our hospital.
During this period, our laboratory processed 75,275 anaerobic blood cultures, yielding 206 anaerobic bacterial isolates, resulting in a positivity rate of 0.3%. These isolates corresponded to 198 patients, aged 15 to 100 years (median: 71 years), with 44.7% being women and 55.3% being men. Isolation involved automated systems, followed by subculturing in anaerobic media and species identification using MALDI-TOF mass spectrometry. Most isolates were Gram-negative (54%) or Gram-positive (36%) bacilli. The predominant pathogens were Bacteroides fragilis (28.6%) and Clostridium perfringens (16%), followed by Eggerthella lenta (5.3%), Fusobacterium nucleatum (3.9%), and Parvimonas micra (2.9%). There were eight cases of mixed infections. The distribution of anaerobic infections followed an expected pattern, with the highest number of cases (104) recorded in the Central Emergency Department, reflecting a large and diverse patient flow.

Conclusion: The findings of this study align with existing literature, confirming that anaerobic BSIs are rare but clinically significant, with Bacteroides fragilis and Clostridium perfringens as the most common pathogens. The data emphasizes the importance of specialized diagnostic techniques and highlights the need for increased clinical awareness and vigilance, particularly in high-risk hospital settings.

P41

EPIDEMIOLOGY AND ANTIBIOTIC SUSCEPTIBILITY PROFILES OF ANAEROBIC BACTERIA IN A PORTUGUESE HOSPITAL

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Introduction: Anaerobic bacteria can cause serious infections and due to their fastidious nature, it is not easy to have an antibiogram in a timely manner. It is therefore important to know the local susceptibility profile for a more effective empirical treatment.

Within this study we aim to analyze the antibiotic susceptibility profiles of anaerobic bacteria isolated in a tertiary hospital in Portugal.

Methods: This study included all non-duplicated anaerobic bacteria isolated in clinical samples during a 21 months period. Identification was performed by MALDI-TOF. Antibiogram were determined with disk diffusion or gradient methods using EUCAST guidelines.

Results: During the period of study, 534 anaerobic microorganisms were isolated. Skin and soft tissue infections were the most common source of isolates (32.2%), followed by intra-abdominal (25.1%) and superior respiratory tract (25.1%), bone and joint infection (8.1%) and blood (7.5%). The most frequent anaerobes were Bacteroides spp (33.5%) followed by Prevotella spp (16.4%) and Fusobacterium spp (15.4%).
For all isolates tested, susceptibility were 97,3% to metronidazole, 72.9% to clindamycin, 72.8% to amoxicillin-clavulanate, 76.9% to piperacillin-tazobactam and 96.6% to meropenem. Among Gram positive anaerobic bacteria, vancomycin was susceptible in all isolates.

Conclusion: Susceptibility testing of anaerobic bacteria and periodic antimicrobial susceptibility surveillance is very important to perceive changes in resistance patterns and to optimize antibiotic therapy.

P42

REASSESSING THE ANALYTICAL PERFORMANCE OF THE STREPTOCOCCUS PNEUMONIAE URINARY ANTIGEN TEST: A 5-YEAR SINGLE-CENTRE RETROSPECTIVE STUDY

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Introduction: Streptococcus pneumoniae (Sp) is a leading cause of community-acquired pneumonia, with traditional culture-based diagnostic methods often exhibiting low sensitivity and requiring 24 to 48 hours for results. In contrast, the Sp urinary antigen (SpUA) test provides results in just 15 minutes, with the patent reporting sensitivities of 86-90% and specificities of 76-94%, using blood culture as a reference standard. However, the test's analytical performance warrants reassessment, particularly using real-world clinical data.

Methods: This single-centre retrospective study examined the results of SpUA tests conducted at a Portuguese university hospital between 1st January 2020 and 31st December 2024, at the request of clinicians. All samples accompanied by blood cultures and/or respiratory samples were included. Sensitivity and specificity were assessed using blood culture or respiratory culture as reference standards.

Results: A total of 1062 SpUA tests were performed, with 116 (11%) yielding positive results. Among the 116 positive SpUA tests, only 21 (18%) were confirmed by positive blood cultures, and 36 (31%) were confirmed by positive respiratory cultures. Furthermore, 8 had negative SpUA results with positive blood cultures, and 35 had negative SpUA results with positive respiratory cultures. When using blood culture as the reference standard, the sensitivity was 72% and specificity was 91%. Using respiratory culture as the standard yielded a sensitivity of 51% and a specificity of 92%. The overall sensitivity was 53%, and the specificity was 92%.

Conclusion: The SpUA test offers a rapid diagnostic alternative to traditional culture methods. However, the lower-than-previously-reported sensitivity suggests a need for reassessment of its clinical utility and its role in diagnostic protocols.

P43

TWO YEARS OF CANDIDA SPECIES EPIDEMIOLOGY AND THEIR SUSCEPTIBILITY TO ANTIFUNGAL AGENTS IN A TERTIARY HOSPITAL IN THE NORTH OF PORTUGAL

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Introduction: Candida species are key pathogens in candidemia, with global shifts in prevalence and antifungal resistance. This study analysed the most common Candida species in a tertiary hospital in northern Portugal.

Materials and Methods: Blood and sterile biological fluid samples collected from 2023 to 2024 were analyzed. Isolates were identified with MALDI-TOF (Vitek® MS V3), and antifungal susceptibility testing (AST) was performed on the first isolate of each sample, using EUCAST guidelines (v10.0, 2020). Antifungals tested included amphotericin B (AB), anidulafungin (A), fluconazole (F), itraconazole (IT), posaconazole (PO), and voriconazole (VO). Results were classified as susceptible (S), susceptible with increased exposure (I), or resistant (R).

Results: A total of 206 Candida spp. isolates from 133 patients (mean age: 63.4 years) were identified. The most prevalent species were C. albicans (41.5%), C. parapsilosis (21.4%), C. glabrata (18.9%), C. krusei (8.3%), and C. tropicalis (5.8%). Other species included C. auris (1), C. dubliniensis (3), C. orthopsilosis (2), C. metapsilosis (1), C. lusitaniae (1), and C. fermentati (1).
Concerning AST, the % of susceptibility for each species-antifungal combination is presented in Table1

	AB		A		F		V		P		IT	
	S	R	S	R	S	R	S	R	S	R	S	R
C albicans	100	0	94,2	5,8	96,5	3,5	96,5	3,5	84,9	15,1	26,7	73,3
C glabrata	100	0	69,2	30,8	*	2,6	ND	ND	ND	ND	ND	ND
C krusei	52,9	47,1	0	100	**	**	ND	ND	ND	ND	ND	ND
C parasilosis	100	0	95,5	4,5	93,2	6,8	97,7	2,3	68,2	22,7	45,5	54,5
C tropicalis	100	0	83,3	16,7	100	0	100	0	66,7	33,3	41,7	58,3

*97,4% I; ** C. krusei has intrinsic resistance to F; ND, no defined CBP

For C. glabrata, the range and modal MICs for V, P, and IT were (0.0125-1, 0.06), (0.0125-2.0, 0.06), and (0.03->4.0, 0.5), respectively. For C. krusei, they were V (0.25-2.0, 0.5), P (0.25-0.5, 0.5), and IT (0.125-4.0, 2.0)

Conclusion: Non-albicans species and multidrug-resistant pathogens like C. auris are emerging, but C. albicans remains predominant in our hospital, showing high susceptibility to antifungals. Understanding the ecology and TSA profiles of Candida species is critical for empirical therapy. Continuous surveillance and appropriate control strategies are essential to prevent antifungal resistance.

P44

INVASIVE PNEUMOCOCCAL DISEASE IN AN UNVACCINATED ELDERLY PATIENT CASE STUDY

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Introduction: Streptococcus pneumoniae (S. Pneumoniae) is a fastidious gram-positive, alpha-hemolytic bacterium, recognized as the main agent of respiratory tract infections.
Despite extensive study of this pathogen and the availability of pneumococcal vaccines, it remains one of the main causes of invasive disease (I.e., bacteremia and meningitis), especially in children and the elderly, with impact on the morbimortality in these age groups.
We report a case of invasive pneumococcal disease in an unvaccinated elderly patient.

Case Description: 80-year-old partially dependent woman was admitted to the Emergency Room due to pre-syncope.
On physical examination she was prostrate, dehydrated, with pulmonary auscultatory alterations, type 1 respiratory failure, hypotensive tension profile and sinus tachycardia. Due to suspicion of sepsis with a respiratory starting point, she was admitted to the Internal Medicine inpatient unit.

The analytical study highlighted: hemogram with marked leukocytosis (47.71/uL) and neutrophilia (92.0%); hypercreatininemia (2.0 mg/dL); increased lactic dehydrogenase (373 mg/dL); hyperphosphatemia (183 mg/dL) and increased C-Reactive Protein (49.65 mg/dL).
All 4 blood cultures were detected as positive 4 hours after collection. Microscopic examination revealed Gram-positive cocci grouped in short chains.
Upon cultural examination alpha-hemolytic colonies were observed, which were identified as S. pneumoniae by Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS Brucker®). Antibiotic Susceptibility Testing was performed in accordance with EUCAST standards and revealed susceptibility to Amoxicillin and Azithromycin, already established as empirical treatment. After treatment for bacteremia, a subcostal empyema was diagnosed, and Ceftriaxone was prescribed. The clinical evolution of this patient had a favorable outcome, without other complications.

Discussion: This case highlights the importance of rapid and accurate diagnosis of invasive pneumococcal diseases in critically ill patients. It also reminds us of the importance of vaccination in this risk group. The identification of S. pneumoniae allowed us to confirm that the instituted treatment was adequate, a sine qua non condition for controlling the focus of infection. Sending the strain for serotyping at the National Reference Laboratory allows for maintaining epidemiological surveillance and adapting the vaccine to the serotypes in circulation.

P45

INVASIVE LISTERIOSIS IN A PATIENT WITH INITIAL DIABETES MELLITUS

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Invasive listeriosis with central nervous system involvement is a severe form of infection caused by the bacterium Listeria monocytogenes. Listeria monocytogenes belongs to the Listeriaceae family and is a Gram-positive, aerobic and facultative anaerobic bacillus that exhibits characteristic motility. It is neither acid-alcohol resistant, non-encapsulated, non-spore-forming. When cultured on blood agar in a capnophilic atmosphere, it produces incomplete beta-haemolysis.

A 41-year-old male patient, independent and cognitively intact, lives in a rural area in a well-maintained residence and denies consuming untreated water.
He was admitted to the Emergency Department with a 5-day history of fever unresponsive to paracetamol and ibuprofen. He reported weight loss, polydipsia and polyuria. Denied recent travel.

Upon physical examination, the patient had elevated blood pressure and fever (38.7°C) with no other significant findings.
Laboratory tests showed leucocytosis (19.58/uL) with neutrophilia (16.3%), hyperglycaemia (376 mg/dL), increased C-reactive protein (25.16 mg/dL) and a urine dipstick showing the presence of ketone bodies, marked haematuria, and proteinuria. Blood cultures were collected, and empirical treatment with ceftriaxone was initiated.
The infectious clinical presentation in the context of newly diagnosed diabetes mellitus led to the Internal Medicine ward.

As his clinical condition worsened, with complaints of neck pain and frontal headaches, a lumbar puncture was performed, which showed pleocytosis (648/uL) and elevated cerebrospinal fluid (CSF) protein concentration (0.92g/L). The bacteriological examination of the CSF was negative. Blood cultures became positive after 19 hours in 3 of 4 bottles, with Gram-positive bacilli observed in the smear. Mass spectrometry (MALDI-TOF MS, Bruker®) identified Listeria monocytogenes. The antibiotic susceptibility testing (Vitek-II, Biomérieux®) revealed sensitivity to ampicillin.
This disease is a nationally notifiable condition and the strain was sent to the national reference laboratory (INSA) for genotyping.

The described clinical case includes bacteremia and meningitis in a patient with risk factors for acquired immunosuppressive conditions predisposing to infection such as diabetes mellitus. The diagnosis was based on laboratory tests.
The rapid diagnosis and early initiation of treatment were crucial for control of the infection focus, glycemic management and ensuring a better outcome.

P46

OVERVIEW OF CLINICAL AND LABORATORY CHARACTERISTICS OF MALARIA PATIENTS

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Introduction: Malaria caused by Plasmodium spp. is a major global infectious disease, making diagnosis and treatment essential for reducing malaria-related morbidity and mortality. A fever in someone who has traveled to a malaria-endemic area should always be promptly evaluated using specific diagnostic tests for malaria. Disease symptoms are related to the parasite species and treatment must be carried out in accordance with this identification. In addition with malaria-specific tests, clinicians should request a complete blood count and a routine chemistry panel. The goal is to detect anemia, hypoglycemia, renal failure, hyperbilirubinemia, and acid-base disturbances.

Aim: This study aims to characterize patients diagnosed with malaria in our hospital from laboratory and clinical perspectives.

Methods: All patients with microbiologically confirmed malaria at our laboratory from 2022 to 2024 were identified and included retrospectively. Demographic data and microbiology, hematology, and biochemistry test results were extracted and analyzed from the laboratory's computer system. Clinical data were retrieved from the clinical computer system and analyzed.

Results: A total of 108 Plasmodium surveys were requested from 88 different patients, and 15 had a positive malaria test (positivity rate of 17%). All positive patients were men, aged between 21 and 55 years (mean age 34.9 years). All patients with a positive test reported fever (15/15), 11 out of 15 reported headaches, and 10 out of 15 reported myalgias.Other symptoms were also reported, including chills, arthralgias, weakness, vomiting, and diarrhea. Two patients were hospitalized (hospitalization rate of 13%), corresponding to the two highest parasitemia values. Thrombocytopenia, which was present in all patients, was more pronounced in these two patients. P.falciparum was identified in most patients (10/15), along with 3 cases of P.ovale, 1 case of P.vivax, and one patient with both P.falciparum and P.ovale.

Conclusions: Clinicians should always obtain a travel history from febrile patients. Microscopic examination remains the gold standard for laboratory confirmation of malaria because it provides species identification, and the percentage of the patient's red blood cells infected with malaria parasites. With a positive malaria test, the results of additional tests will help determine whether the patient has an uncomplicated infection or one with severe manifestations.

P47

FIRST CASE REPORT OF A NEW DELHI METALLO-B-LACTAMASE PRODUCER STRAIN OF KLEBSIELLA AEROGENES IN A LOCAL HEALTH UNIT

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Introduction: Antibiotic resistance is a serious threat to global health, particularly from carbapenemase-producing bacteria. According to ECDC, resistance to carbapenems in K. pneumoniae isolates in Portugal increased 43% (2019-2023, EU average 57.5%).
New Delhi Metallo-β-lactamase (NDM) producer strains are of particular concern because they exhibit high resistance to most antibiotics, which significantly limits therapeutic options.

Materials and Methods: A 63-year-old male, living in a long-term care facility, totally dependent at activities of daily living, with nasogastric and bladder catheters, history of alcohol/tobacco abuse, several cardiovascular, metabolic, respiratory and neurologic comorbidities, experienced multiple hospitalizations (August to December 2024) due to recurrent urinary tract infections (UTI), comorbidities exacerbation and infected bedsores.
Uroculture/isolation: MacConkey and CLED agar mediums (bioMérieuxTM).
Identification: colorimetry/turbidimetry biochemistry assays (VITEKTM 2 Compack, bioMérieux).
Antimicrobial susceptibility testing (AST): serial antibiotic dilutions (VITEKTM 2 Compack, bioMérieux). Ceftazidime/avibactam, azetreonam, ertapenem: antibiotic gradient ETESTTM (bioMérieux). Colistine: broth microdilution (UMICTM Colistin, BRUKER).
Carbapenemase phenotyping: carbapenem hydrolysis (chromogenic tests RAPIDECTM CARBA NP - bioMérieux).
Genotyping: RT-PCR (XpertTM Carba-R, Chepheid).

Results: We isolated an NDM-producing Klebsiella aerogenes. AST were performed according to EUCAST guidelines, and the MIC obtained were ceftazidime/avibactam >256µg/dL, ertapenem 8µg/dL, and aztreonam 16µg/dL. The patient died of sepsis a few days after hospitalization.

Discussion: Although our microbiology laboratory has detected multiple NDM-producing strains in external quality assessments, this is the first clinical report within our local health unit. Given the patient's multiple risk factors for infection with carbapenemase-producing Enterobacterales, according to national health authorities' guidelines, a screening through rectal swab should have been performed.

Conclusion: NDM-producing strains have emerged since 2018, highlighting the need for microbiology laboratories in local health units to implement rapid isolation and identification methods along with molecular biology techniques for genotypic detection, which is crucial to improve treatment success and prevent the spread at hospital environments and in the community.

P48

CORYNEBACTERIUM UREALYTICUM: A CLINICAL CASE OF URINARY TRACT INFECTION

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Introduction: Corynebacterium urealyticum (CU) is a recognized pathogen responsible for urinary tract infections (UTIs), often seen in patients with comorbidities such as tumors and inflammation.

CU is an opportunistic nosocomial pathogen associated with conditions like cystitis, alkaline encrusted cystitis, pyelonephritis, and encrusted pyelitis, and it may also lead to bacteremia, particularly in patients with chronic urological disorders. CU is a gram positive, slow-growing, lipophilic, urease positive microorganism with diphtheroid morphology.
The majority of CU currently isolated from clinical samples are multidrug resistant, thus potentially limiting effective empirical treatment.
Accurate identification and appropriate therapy are essential to prevent avoidable complications. We report a case of C. urealyticum UTI in a man with some comorbidities, with the microorganism isolated from a urine sample.

Case description: An 88-year-old male with a history of prostate cancer and recurrent UTIs presented to the emergency department with urinary complaints.
The urine culture revealed pure growth of tiny colonies on blood agar and CLED agar after 24 hours incubation (positive ≥104 UFC/mL).
The isolate was successfully identified as CU by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry (MALDI Biotyper®, Bruker, Germany).
Antibiotic susceptibility was performed too, and the organism was susceptible to vancomycin, linezolid, tetracycline, rifampicin and clindamycin, but resistant to ciprofloxacin and penicillin.

Discussion: This case highlights the importance of recognizing CU as a potential uropathogen, particularly in patients with comorbidities and/or chronic urological disorders.
The slow growth of CU during overnight incubation can lead to its underrecognition in routine cultures, making accurate identification through MALDI-TOF mass spectrometry essential for improving diagnostic.
Emphasizing the importance of early recognition, this case underscores the need for increased awareness of CU in clinical settings and the critical role of advanced identification techniques to enable timely and appropriate treatment, thereby preventing complications in patients with underlying comorbidities.

P49

DIAGNOSIS OF A ZOONOTIC INFECTION – PASTEURELLA MULTOCIDA

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Introduction: Pasteurella multocida is a Gram-negative facultative anaerobe coccobacillus, that typically grows well on blood agar and chocolate agar but has a great difficulty in growth on MacConkey agar.
It is generally sensitive to penicillin and can be associated with skin infections in humans resulting from bites or scratches from domestic animals, mainly dogs and cats, as it is part of the normal respiratory microbiota.
Respiratory or invasive clinical manifestations such as bacteremia, meningitis, and endocarditis are rarer but can occur, particularly in elderly, immunocompromised individuals, and neonates.

Case Description: An 81-year-old man presented to the emergency department with fever, dyspnea and cough with sputum for four days, without other significant complaints. On physical examination, he was febrile (38.2ºC), tachycardic, and had mucopurulent sputum. His relevant medical history included chronic kidney disease, hypertension, and atrial flutter.

Laboratory findings revealed leukocytosis ($15.1\times10^3/\mu L$) and elevated C-reactive protein (CRP) (15.02 mg/dL). Urine culture, sputum culture, and blood cultures were collected, with the latter turning positive within 24 hours.
The presence of Gram-negative coccobacillus on the Gram-stained smear and the growth of large, mucoid, grayish, non-hemolytic colonies on blood agar but no growth on MacConkey agar suggested possible Pasteurella isolation.
The diagnosis was confirmed through biochemical identification using the Vitek®2 system.
Antimicrobial susceptibility testing revealed a multi-sensitive strain of Pasteurella multocida.
Based on these results, the empiric therapy with Ceftriaxone and Azithromycin was maintained.
After 15 days of Ceftriaxone and 3 days of Azithromycin, the patient showed clinical improvement.

Discussion: Bacteremia caused by Pasteurella multocida is rare. The described case represents the 6th isolation in our hospital in 10 years.
In this case, although no clear entry point was identified, close contact with a house cat might have been the source of infection.
We believe that the patient's advanced age and comorbidities were key factors in the development of bacteremia. Early definitive identification and the implementation of appropriate treatment were essential for a favourable clinical outcome.

P50

TRACKING SHIGELLA: A RETROSPECTIVE LOOK THROUGH A TERTIARY HOSPITAL ISOLATES

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Introduction: Shigellosis is a gastrointestinal infection caused by Shigella species, leading to symptoms that range from mild diarrhea to severe dysentery. Humans are the only known reservoir, and transmission primarily occurs through the fecal-oral route. High-risk groups include young children, international travelers, and men who have sex with men.
Four Shigella spp. have been identified: S. sonnei (common in adults in developed countries), S. flexneri (prevalent among children), S. dysenteriae (which causes severe cases), and S. boydii (less common). The increasing number of cases and the growing antimicrobial resistance pose significant public health challenges.

Description of Casuistry: We conducted a retrospective observational study on culture-positive Shigella spp. results from stool samples collected between 2014 and 2024. Among 14,716 processed samples, 46 (0.3%) tested positive for Shigella spp., indicating a significant increase in the positivity rate in 2024 (25% of the total). This included 43 patients aged 1 to 76 years, with a median age of 34 and a male predominance of 90.7%. Shigella spp. were isolated using selective and differential agar media. Identification and antimicrobial susceptibility testing (AST) were performed using automated broth microdilution, and serotyping was conducted through slide agglutination. The most frequently reported species were S. flexneri (n=26), followed by S. sonnei (n=15) and Shigella sp. (n=2). Most isolates originated from the emergency department. Overall AST (n=44) indicated resistance rates of 40.9% to ciprofloxacin, 52.6% to amoxicillin/clavulanate, 79.5% to ampicillin, and 56.8% to cotrimoxazole, with increased resistance noted in S. sonnei isolates (n=15), which demonstrated 80% resistance to ciprofloxacin and 86.7% to cotrimoxazole.

Discussion: Shigellosis remains a significant global public health concern, with S. flexneri commonly found in developing countries and S. sonnei more prevalent in developed nations. Our study indicates a considerable increase in cases in 2024, reflecting global trends. Although it is a reportable disease, Portugal lacks current data for comparison. Our findings emphasize the need for culture-dependent testing in surveillance and highlight the growing challenge of antimicrobial resistance.

P51

ANTIMICROBIAL RESISTANCE PROFILE OF ESCHERICHIA COLI – A COMPARATIVE STUDY IN A LOCAL HEALTH UNIT

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Introduction: Antimicrobial resistance is a major public health concern, compromising antibiotic efficacy and increasing infection-related mortality.
Different healthcare settings influence resistance patterns due to variations in antibiotic use, exposure duration and patient characteristics.

Aim: This study compares the antimicrobial resistance profile of Escherichia coli isolated from urine cultures of hospitalized, outpatient and institutionalized patients in a Local Health Unit, aiming to improve the understanding of resistance dissemination and support antibiotic stewardship and infection control strategies.

Methods: A six-month retrospective study (January – June 2024) analyzed 785 positive urine cultures from hospitalized, outpatient and institutionalized patients. Data were collected from the LIS Modulab system, including only urine cultures with E. coli isolates.

Results: E. coli was the most prevalent pathogen (59%), followed by Klebsiella pneumoniae (16%).
Resistance rates varied across patient groups. Resistance to amoxicillin-clavulanic acid was 30.1% in outpatients, 46.0% in hospitalized patients and 36.7% in institutionalized patients. Ciprofloxacin resistance was 26.2% in outpatients, 50.0% in hospitalized patients and 83.3% in institutionalized patients.
Cotrimoxazole resistance was 25.9% in outpatients, 30.0% in hospitalized patients and 50.0% in institutionalized patients. Fosfomycin resistance was 6.5% in outpatients, 4.0% in hospitalized patients and absent in institutionalized patients. Nitrofurantoin resistance was 0.3% in outpatients and 0.0% in both hospitalized and institutionalized patients.

Conclusions: Outpatients had E. coli isolates with lower resistance rates compared to hospitalized and institutionalized patients, as expected.
E. coli isolates from institutionalized patients exhibited the highest resistance rates, exceeding those in hospitalized patients. This difference was most pronounced for ciprofloxacin (83.3% vs 50.0%) and cotrimoxazole (50.0% vs 30.0%).
The low resistance to nitrofurantoin and fosfomycin highlights their importance for treating uncomplicated urinary tract infections, particularly for multidrug-resistant strains.

These findings underscore the urgent need for improved antimicrobial resistance surveillance and control strategies in institutionalized patients, emphasizing antibiotic stewardship and the implementation of stricter infection control measures.

P52

TOXOPLASMOSIS IN PREGNANCY: THE PREVALENCE OF ANTI-TOXOPLASMA GONDII ANTIBODIES AMONG PREGNANT WOMEN AT A COMMUNITY LABORATORY IN 2024

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Background: Toxoplasmosis is an infectious disease caused by the obligate intracellular protozoan Toxoplasma gondii.
It can be acquired by ingesting water or food contaminated with oocysts from the soil or by consuming tissue cysts in raw or undercooked meat. Most healthy individuals infected with toxoplasmosis show no signs or symptoms and remain unaware of their infection. However, if contracted during pregnancy, it can have serious consequences for the fetus, with the severity depending on the timing of the pregnant woman's exposure to the parasite.
According to DGS Standard 037/2011, dated 30/09/2011, all pregnant women with an unknown serological status for toxoplasmosis should be screened during their pregnancy, and if the results are negative, they should repeat the test in the second and third trimesters. The prevalence of seropositivity in women of reproductive age was estimated at 18% in 2013. There is limited data regarding the immunological status of toxoplasmosis in Portugal.

Aim: Evaluate the prevalence of anti-Toxoplasma gondii antibodies among pregnant women monitored in our laboratory during 2024. Additionally, we seek to estimate the seroprevalence of this infection to reduce the risk of transmission to the child.

Results: We studied 390 pregnant women aged 18 to 50 years (average: 31.4; median: 31). Among the participants, 86.3% tested negative for IgG and IgM anti-Toxoplasma gondii, while 13.7% tested positive for IgG and negative for IgM anti-Toxoplasma gondii. In our study, the prevalence of antibodies was 11.5% in women under 30 years old, compared to 15.2% in those over 30. Additionally, we found that 48.6% of the women with positive IgG anti-Toxoplasma gondii in our study were foreign-born.

Conclusion: The prevalence of toxoplasmosis-specific antibodies in the studied population was low (13.7%), indicating that the measures implemented to reduce the risk of transmission to the child are effective and should be maintained during pregnancy.
The fact that 48.6% of women with positive serology were foreign suggests that the measures in other countries may not be as effective as those in Portugal. We observed that the prevalence is higher among older women.
According to Gargaté et al. (2016), the age of pregnant women is directly proportional to the prevalence of specific antibodies to toxoplasmosis, as the likelihood of exposure to parasites increases over time.

P53

HEPATITIS A IN PEOPLE LIVING WITH HIV: THE IMPORTANCE OF VACCINATION IN PREVENTING COMPLICATIONS

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Introduction: Hepatitis A is an acute liver infection caused by the hepatitis A virus (HAV), primarily transmitted via the faecal-oral route. The incubation period ranges from 28 to 30 days. While often asymptomatic, the disease can be severe in immunocompromised individuals, including people living with HIV (PLWH). Vaccination is the most effective preventive measure, providing long-term immunity with a well-established safety profile.

Case Description: We present the case of a 36-year-old man with HIV-1 who presented to the emergency department with nausea, vomiting, myalgia, fever, dark urine, and pale stools.
On examination, he exhibited jaundice and significant liver enzyme abnormalities, with elevated transaminases and bilirubinuria. Serology confirmed acute hepatitis A. Despite prior medical recommendations, he had not been vaccinated. His hospitalisation was prolonged due to immune dysregulation, leading to a delayed recovery.

Discussion: There is no specific treatment for hepatitis A, and in PLWH, symptom resolution may be prolonged, with an increased risk of hepatic complications, including potential toxicity from antiretroviral therapy. Studies indicate that vaccinated individuals develop milder disease or avoid infection entirely. In this case, the lack of vaccination contributed to a more severe outcome, highlighting the importance of immunisation in at-risk populations. Expanding vaccine coverage is essential to reducing morbidity and healthcare costs.

Relevance: HAV is highly contagious, and vaccination not only prevents infection but also contributes to herd immunity. The Portuguese Directorate-General of Health (DGS) recommends pre-exposure vaccination for PLWH, men who have sex with men, and travellers to endemic areas. Expanding free vaccine access for these groups is a crucial public health measure.

Conclusions and Lessons Learned:

1. Hepatitis A can be severe in PLWH, increasing hospitalisation rates.
2. Vaccination significantly reduces disease severity and the risk of complications in immunocompromised individuals.
3. Public health policies should enhance vaccine accessibility to prevent outbreaks and optimise healthcare resources.
4.

P54

CHARACTERIZATION OF BAAR POSITIVITY IN RESPIRATORY SAMPLES: 2014-2024

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Introduction: Tuberculosis is an infectious disease caused by bacteria of the Mycobacterium tuberculosis complex.
In 2023, it is estimated that 10.8 million people will have the disease, and in 2022, 1518 cases were reported. Epidemiological control of tuberculosis is fundamental to maintaining the downward trend in incidence that has been seen in recent years. Despite the development of new laboratory diagnostic methods, testing for acid-fast bacilli (BAAR) is still the initial method when infection is suspected. We analyzed the incidence of positive BAAR tests over a 10-year period in a district hospital.

Objectives and Methodology: This was a case-based, descriptive study of the incidence of BAAR in respiratory samples from 01/01/2014 to 31/12/2024. CLINIDATA® XXI was used to collect all requests for BAAR testing in emergency and non-urgent settings during the period indicated. Microsoft Excel was used for statistical analysis.

Results: During the period in question, 4415 patients were studied, with a total of 11722 requests for BAAR testing, of which 2002 (17%) were in an emergency setting, and 9720 (83%) in a non-urgent setting. The services with the most requests were Pulmonology, Infectious Diseases and the ER.
Of the total number of BAAR requests, 1009 (8.6%) were positive, corresponding to 214 patients, and the overall positivity per patient was 4.8%. In 2024, the figure was 3.81%.
Of the 214 patients with a positive BAAR test, 74.5% were male and 25.5% female, with an average age of 48.2, with 79.3% aged between 20 and 65.

Discussion: The number of tuberculosis cases in Portugal has been falling in recent years, and in 2015 reached the threshold considered to be low incidence (20 cases per 100,000 inhabitants per year). However, the incidence rate in Portugal remains one of the highest in the European Union, with a value of 13.4 in 2022, compared to 8.6 in the EU.
BAAR testing continues to be a fundamental tool in the epidemiological control of tuberculosis, with the laboratory and clinical pathologist playing a central role in this identification, allowing for faster treatment and preventing the appearance of new cases.

P55

HEPATITIS E VIRUS: THE NEED FOR BETTER SURVEILLANCE, DIAGNOSIS, AND PREVENTION

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Introduction: Hepatitis E virus (HEV) infection is a leading cause of acute viral hepatitis globally, with significant morbidity and mortality, particularly in endemic regions.
Transmission occurs mainly via the fecal-oral route through contaminated water, but zoonotic and parenteral transmissions have been reported.

Objective: This study aims to analyze the epidemiology, diagnostic challenges, and public health implications of HEV infection, highlighting preventive and control strategies.

Materials and Methods: An integrative review was conducted using databases such as PubMed, Scopus, and Web of Science during january 2025.

The search strategy focuses on the main concepts: Hepatite E, epidemiological, diagnostic methodologies and clinical outcomes, with results limited to studies published between 2010 and 2024.

Results: A total of 18 studies were included. In these studies, underlines HEV is responsible for sporadic and epidemic outbreaks, with genotypes 1 and 2 predominantly affecting developing countries, while genotypes 3 and 4 are linked to zoonotic transmission in industrialized nations. Diagnostic methods include serological assays for anti-HEV antibodies and molecular techniques for viral RNA detection.
Despite the availability of a vaccine in China, global immunization efforts remain limited. The underdiagnosis of HEV due to nonspecific symptoms and limited routine testing poses a challenge for disease control. Immunocompromised individuals and pregnant women face higher risks of severe outcomes, necessitating targeted interventions. Improvements in sanitation, water quality, and surveillance are crucial for reducing HEV burden.

Conclusion: This review shows that HEV remains a global health concern, requiring enhanced diagnostic strategies, vaccination policies, and preventive measures. Greater awareness and public health initiatives are essential to mitigate its impact.

P56

TRENDS IN CLOSTRIDIOIDES DIFFICILE INFECTION: FIVE-YEAR PREVALENCE STUDY

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Introduction: Clostridioides difficile infection (CDI) is a leading cause of diarrhea in hospital and long-term care settings.
Transmission occurs via the fecal-oral route, leading to illness when gut microbiota is disrupted. Key risk factors include recent antibiotic use, aging, immunodeficiency and hospitalization.

Objective: Evaluate the prevalence of C. difficile over a five-year period in outpatients and inpatients.

Material and Methods: Detection of C. difficile by Immuno Card™ C. difficile GDH (Meridian Bioscience™), an immunochromatographic assay detecting C. difficile glutamate dehydrogenase antigen (GDHa) and Alethia™ C. difficile Assay (Meridian Bioscience™), a Loop-Mediated Isothermal Amplification assay targeting the cytotoxin gene.
Data collection: ModuLab software™ (Werfen™).
Statistical analysis: Microsoft Excel™.
Population: 1582 samples from inpatients and outpatients from January 2019 to December 2023 in two hospitals (1 and 2).
Rejection criteria: Samples without loose stool or those collected from the same patient within one year.

Results: Of the 1582 samples, 986 were accepted for C. difficile GDHa testing, with 135 (13.7%) testing positive. The cytotoxin gene assay was performed on these 135 samples, confirming 96 (71.0%) as positive. Among them, 90 (93.8%) were patients over 65 years old.

In Hospital 1, 50 (67.0%) inpatients and 31 (76.0%) outpatients tested positive, while in Hospital 2, 15 (83%) of GDHa-positive inpatients carried the cytotoxin gene. We obtained a median hospital stay of 12 days for inpatients in both settings. Cases of cytotoxin increased over time: 15 (15.7%) in 2019, 11 (11.5%) in 2020, 20 (20.8%) in 2021, 25 (26.0%) in both 2022 and 2023, with 2020's drop linked to COVID-19.

Discussion: As reported, increasing age is a major CDI risk factor, reflected in our findings. Prolonged hospitalization adds risk, with most cytotoxin-positive cases observed in inpatients. The increasing numbers suggest an emerging trend. Additionally, the detection of the cytotoxin gene in most GDHa-positive samples reinforces the importance of molecular testing as a complement to immunochromatographic assays in identifying toxigenic C. difficile strains.

Conclusion: The increasing CDI incidence highlights the need for vigilance and enhanced infection control measures.

P57

PREVALENCE AND CLINICAL CHARACTERIZATION OF MYCOBACTERIUM CHELONAE IN A TERTIARY HOSPITAL: A FOUR-YEAR STUDY

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Introduction: Nontuberculous mycobacteria have gained increasing clinical relevance, especially in patients with predisposing conditions such as immunodeficiency, cystic fibrosis (CF) or bronchiectasis (BC). Among these, Mycobacterium chelonae (MC) stands out due to its rapid growth rate and ability to cause chronic pulmonary, cutaneous or disseminated infections.
In recent years, our hospital has observed an apparent increase in the identification of MC, particularly in at-risk groups. This study aims to analyze epidemiological data from the past four years, characterize the profile of affected patients, and explore possible factors associated with the observed increase.

Case Description: A retrospective analysis of 28 cases of MC identified between 2020 and 2024 was conducted. Patients had a mean age of 67 years (range: 22–91), with a male-to-female ratio of 17:11. The Pulmonology Outpatient Clinic was the most frequent requesting service, and bronchiectasis was the predominant underlying condition. The number of cases increased over the years (1 in 2020, 1 in 2021, 4 in 2022, 11 in 2023, and 11 in 2024). The most common sample type was sputum.
Of five requested antimicrobial susceptibility tests (TSA), three were viable, revealing resistance to Sulfamethoxazole and sensitivity to Amikacin, Clarithromycin, Moxifloxacin, and Linezolid.
Diagnosis was confirmed through Ziehl-Neelsen staining, culture, and molecular identification using the GenoType Mycobacterium CM (reverse hybridization) was employed to identify clinically relevant mycobacterial species, ensuring precise species differentiation.

Discussion: The results of this study show a consistent increase in the number of MC identifications over the past four years.
This growth may be related to various factors, including greater clinical awareness and changes in epidemiological patterns. The most affected patients were those with chronic respiratory conditions and immunocompromised.

P58

ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF AEROMONAS ASSOCIATED WITH BLOODSTREAM INFECTION

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Introduction: Aeromonas bloodstream infections are relatively rare but have the potential to cause severe outcomes. These microorganisms are often reported as susceptible to third-generation cephalosporins.
However, they have a notable tendency to produce chromosomally encoded beta-lactamases, which may compromise the effectiveness of antimicrobial treatments.

Objectives: This study aims to evaluate antimicrobial resistance profiles in bloodstream infections caused by Aeromonas.

Materials and Methods: We conducted a retrospective analysis of blood culture results processed between January 2016, and December 2023, at a clinical microbiology laboratory in Portugal.

Results: During the study period, 23 episodes of Aeromonas bacteremia were identified, 65% of which occurred in men and 87% in individuals aged 65 years or older. The most common associated clinical conditions were hepatobiliary-pancreatic disorders. Most infections were community-acquired, and 39% had polymicrobial bacteremia.
The mean time to blood culture positivity was 9.8 ± 2.3 hours. When tested, the isolates were all susceptible to ciprofloxacin (n=19), ceftazidime (n=19), trimethoprim-sulfamethoxazole (n=15), cefepime (n=13), aztreonam (n=6), and levofloxacin (n=12).

Conclusions: We found a low incidence of Aeromonas isolates resistant to the antibiotics tested.

P59

STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS - THE IMPORTANCE OF ANAMNESIS

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Clinical Case Summary: Streptococcus equi subsp. zooepidemicus - The importance of anamnesis.

Introduction: Streptococcus equi subsp. zooepidemicus is a zoonotic pathogen associated with respiratory infections in horses. Human infections, though rare, can be severe, leading to bacteremia, endocarditis and other diseases.
Transmission occurs through direct contact with infected animals, particularly horses, or via consumption of contaminated unpasteurized milk or dairy products.
The identification of this bacterium in human infections is clinically significant, as its manifestations can be easily mistaken for infections caused by other streptococci.

This case highlights the critical role of collaboration between the laboratory and the clinicians in accurately diagnosing S. equi subsp. zooepidemicus in a human patient.

Objective: To describe a case of mitral valve endocarditis caused by S. equi subsp. zooepidemicus and emphasize the importance of laboratory-clinician communication for accurate pathogen identification.

Clinical Case: An 83-year-old male with chronic alcoholism and coagulopathy presented with fever of unknown origin. Cardiac auscultation suggested valvular disease, and echocardiography revealed mitral valve vegetations, raising suspicion of infective endocarditis.
Anamnesis revealed frequent contact with goats and donkeys due to farm work.
Three sets of blood cultures were positive within 48 hours. Gram staining showed Gram-positive cocci in chains. Culture on blood agar under aerobic and anaerobic conditions demonstrated the growth of large, mucoid colonies with a beta-hemolytic halo. The pathogen was identified using the Vitek® 2 compact system.

Discussion/ Conclusion: Identifying S. equi subsp. zooepidemicus in human infections is challenging due to phenotypic similarities with other streptococci.
In this case, close collaboration between the laboratory and clinicians was crucial for confirming the pathogen and establishing the diagnosis.
The diagnostic approach integrated laboratory findings and anamnesis, identifying occupational exposure as a risk factor. This case emphasis the need for a multidisciplinary approach and the importance of considering zoonotic infections in endocarditis differential diagnosis, particularly in patients with known risks.

P60

PIVMECILLINAM IN STAPHYLOCOCCUS SAPROPHYTICUS URINARY INFECTION?

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Introduction: Urinary tract infections (UTIs) are the most common bacterial infections. Although UTIs can be associated with serious illness, most cases are considered uncomplicated UTI.
Pivmecillinam, an orally active prodrug of Mecillinam (MEC), has been used for the treatment of uncomplicated UTIs since the 1970s in Northern European countries, demonstrating clinical efficacy and low resistance rates (4-6%).
Staphylococcus saprophyticus (responsible for about 10% of UTIs) is considered resistant in vitro to MEC, with no defined breakpoints and with only minimal inhibitory concentration (MIC) observations. Nevertheless, studies have shown high clinical efficacy, as this drug reaches high concentrations in urine.

Objective: This study aimed to determine S. saprophyticus sensibility to MEC and assess whether it is a viable oral therapeutic alternative.

Materials and Methods: A prospective study was conducted from November 2024 to January 2025 to determine the sensitivity of S. saprophyticus strains isolated from urine samples of community patients with UTIs to MEC.

All isolates were identified using an automated identification system (VITEK MS®, bioMérieux). MEC susceptibility (10mg) was determined by the disc diffusion method.

Results: A total of 41 isolates were studied, with the majority being from women (n = 39; 95%) and a median age of 36 years. Of the strains studied, 36 showed no inhibition zone (<= 6mm) and 5 showed inhibition ranging from 7-14mm.

Conclusion: According to European and American guidelines, EUCAST and CLSI, respectively, there are no defined breakpoints to determine the in vitro sensitivity of S. saprophyticus to MEC.
However, studies show that the concentration of MEC in urine reaches levels of 200mg/L, making the treatment of UTIs caused by S. saprophyticus with this drug effective and considering this antibiotic a oral therapeutic alternative to Fosfomycin to wich S. saprophyticus is intrinsically resistant. Therefore, clinical studies with MIC data are needed to examine potential breakpoints and correlate laboratory results with clinical outcomes.

P61

SPOROTHRIX MOLECULAR DETECTION

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Sporotrichosis, caused by the dimorphic fungus Sporothrix, occurs by traumatic inoculation (cutaneous or subcutaneous form) or inhalation of conidia (extracutaneous form).
These fungi are saprophytes in vegetation, organic matter and soil. Although rare, some cases of zoonotic transmission have been described. (1,2)
The most clinically relevant species are S. brasiliensis, S. schenckii, S. globosa and S.luriei.(2)
The gold standard diagnosis is culture, but advances in molecular diagnostics have brought faster diagnosis, allowing specific treatment, having a positive impact on recovery and reduction in transmission rates. (2,3)

The choice of treatment depends on the clinical form, the patient's immune status and the species involved. The most commonly used antifungal is itraconazole and treatment lasts 3-6 months or up to 4-6 weeks after complete clinical remission. (3,4)
Here we present a molecular technique that allows the detection of this fungus, based on Sybrgreen ® real time PCR. The primers, adapted from Hu et al (5)., amplify a conserved region of the 18S rRNA gene, allowing rapid and specific detection directly from samples such as pus, abscesses and skin biopsies. DNA was extracted from samples, after digestion with proteinase K and liticase, in a Qiamp ® DNA protocol. (5)

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P62

A CASE OF A PATIENT WITH STAGHORN CALCULI LOST TO FOLLOW-UP

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Introduction: Nephrolithiasis is a common disease, lifetime prevalence in Portugal is estimated at 7.3%. About 7-8% of all cases are struvite stones, caused by repeated upper urinary tract infections by urease-producing bacteria. The accumulation of ammonia leads to the formation of struvite stones, composed of calcium magnesium ammonium phosphate and carbonate apatite. These stones can reach great dimensions – hence the name staghorn calculi – and lead to obstruction of the urinary tract. Treatment in such instances is surgical, untreated patients may suffer life-threatening complications. Recurrence is frequent.

Case presentation: A 44-year-old female patient with a history of percutaneous nephrolithotomy at age 24, lost to follow up since, presented to Emergency Department (ED) with asthenia, anorexia, significant weight loss (24 kg in four years) and amenorrhoea. Analytically showed microcytic anaemia, leucocytosis and renal failure (Creatinine 2,53 mg/dL). CT Imaging described large, bilateral staghorn calculi filling the totality of the pyelocaliceal system of both kidneys, causing severe obstruction. Was subjected to open nephrolithotomy of the left kidney, extracting a 386g calculus. Analytically, the stone was composed of triphosphate. Returned to the ED with fever, severe lower back pain and hyponatraemia and was admitted with the diagnosis of sepsis secondary to pyelonephritis caused by Enterococcus faecalis. The patient was subject to left urethral catheterization and right nephrectomy. Histological examination of the right kidney revealed the presence of high-grade, invasive urothelial carcinoma, undetected in prior imaging scans. Despite adequate intensive care, the patient died ten days after the procedure.

Discussion: Our case underscores the importance of proper follow-up in case of struvite stones. These have a high risk for recurrence and pose serious life-threatening risks, such as urosepsis. In addition, upper tract urothelial carcinoma risk is higher in patients with kidney stones, especially those that have an early kidney stone diagnosis (≤40 years).
Follow-up should be maintained with an urologist with periodic screening through laboratory exams and imaging. Early diagnosis and treatment of recurrence improves outcomes and may prevent complications, including sepsis or carcinoma.

P63

COMPARISON OF FAECAL CALPROTECTIN QUANTIFICATION BY IMMUNOTURBIDIMETRIC METHODS IN TWO AUTOMATED ANALYSERS

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Introduction: Faecal calprotectin (FC) is considered a good non-invasive marker for inflammatory bowel disease diagnosis and disease activity.

Objective: In our laboratory, FC levels are measured using an immunoturbidimetric methodology on the INTEGRA 400 plus analyser (Roche Diagnostics). Due to work overload, there is a need to incorporate new analysers into the laboratory routine to optimise workflow. This study aimed to compare FC quantification between the INTEGRA 400 plus, used as the comparative analyser (X), and the Respons®910 DiaSys (Diagnostic Systems GmbH), the test analyser (Y), applying the EP09 protocol (CLSI).
The accuracy and stability of the INTEGRA 400 plus for FC quantification have been confirmed by several studies. However, there are few studies on the Respons®910 DiaSys analyser.

Methods: Stool samples were obtained from patients attending inflammatory bowel disease clinic appointments. Duplicates were collected from 31 stool samples for FC quantification.
The Pearson correlation coefficient (R) was calculated to determine whether the number of samples was adequate to compensate for the error in the Y method, with 0.95 as the threshold set by the EP9 protocol as an acceptable data range to compensate for the error in the test method. The Shapiro-Wilk test (α=0.05) was used to assess data normality.
The Bland-Altman analysis was used to evaluate differences and bias between FC measurements from both analysers.

Results: The data followed a normal distribution, with a Shapiro-Wilk test p-value of 0.28. The R correlation coefficient was 0.61 (<0.95), and the Bland-Altman analysis showed a negative average bias of approximately -44 for Y method measurements.

Conclusion: An R value of 0.61 suggests a positive moderate correlation of X and Y measurements meaning that measurements are related, but with significant variability. The Bland-Altman analysis showed a non-linear relationship between the measurements from both analysers, with a bias of approximately -44. This may indicate issues with measurement consistency, biological variability, or systematic differences between the two methods. These findings may be partially explained by the heterogeneous distribution of FC in stool, leading to high within-sample variability of FC content. Additional FC measurements are needed to address within-sample variability and measurement error in the Y method before considering its future implementation in the laboratory routine.

P64

CARBOHYDRATE ANTIGEN 125 AND BLOOD NATRIURETIC PEPTIDES IN THE ASSESSMENT OF HEART FAILURE: ARE THEY COMPLEMENTARY OR INDEPENDENT?

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Introduction: Natriuretic peptides (NP) such as BNP and NT-proBNP are widely used in the assessment and management of heart failure (HF). Carbohydrate Antigen 125 (CA125) is traditionally used as a tumor marker, but recent studies suggest its usefulness as a promising complementary biomarker in different HF scenarios, proving to be elevated in congestion, inflammation, disease severity and worse prognosis. In acute HF, serum CA125 levels were more significantly associated with a state of congestion than NT-proBNP. Following this association, the authors explore the correlations between NP and CA125 in the context of HF in a retrospective analysis.

Objective: Assess the correlation between CA125 and the NP – BNP and NT-proBNP – in a one-year retrospective analysis, exploring the context of complementarity of these biomarkers in HF.

Methods: A retrospective analysis was conducted using one-year results of CA125 and NP. Only samples with CA125 values above the cutoff threshold (> 27 U/mL) were included, using the result for BNP or NT-proBNP in the same day. Samples from the oncology department were also excluded. The samples were processed on the Alinity i® analyzer (Abbott®) for all biomarkers. Microsoft Excel® was used for statistical analysis of the Person's correlation.

Results: A total of 227 CA125 results were analyzed, combined with 61 BNP and 166 NTproBNP. Mean, minimum and maximum results were: 94.676/ 4.100/ 2,975.900 U/ml for CA125; 775/ 9/ 13,175 pg/ml for BNP and 3,024/ 23/ 106,526 pg/ml for NTproBNP. A positive linear moderated correlation was found between CA125 and BNP (r=0.603) and a positive linear weak correlation was found between CA125 and NTproBNP (r=0.130). Higher CA125 levels were also associated with worse prognosis, including death.

Conclusion: This retrospective analysis suggests that CA125, alongside NP, provides valuable insights in HF patients. CA125 correlates better with BNP than with NTproBNP, in HF conditions, with the higher values being close to the severity and poorer prognosis, corroborating with the literature. To answer the question of complementarity or independence, additional prospective studies will be necessary, but a strong prognostic value appears to be associated with CA125 in the management of patients with FH. The inferior number of associations between the CA125 results and BNP (27%, compared to NTproBNP) may constitute a limitation of the study.

P65

ASSESSING HEMOGLOBIN LEVELS IN HUMAN STOOL SAMPLES FOR COLORECTAL CANCER SCREENING

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Introduction: A population-screening program for the detection of precancerous lesions reduces de incidence and mortality of colorectal cancer (CRC). Fecal immunochemical tests (FIT) are stool-based tests, for the detection of human hemoglobin. FITs are cost-effective and non-invasive with a sensitivity of ≈73% and specificity of ≈96%.
Qualitative immunochromatographic FITs (QlFITs) provide positive or negative results, whereas quantitative FITs (QtFITs) offer automated analysis, yielding numerical values that enhance consistency and allow cut-off adjustment based on patient risk for CRC.

Objective: In our clinical chemistry laboratory, we use a qualitative stool-based FIT in the screening for CRC and intend to implement an automated QtFIT. So, we compared the diagnostic performance of both QlFIT and QtFIT, with a threshold for human hemoglobin detection of 6µg/g and 1µg/g, respectively.

Materials and Methods: A total of 32 stool samples were analyzed using the QlFIT (Fecal Occult Blood Rapid Test, Healgen®) and the QtFIT (IDK® TurbiFIT®, Immundiagnostik AG). QlFIT results were visually interpreted as either positive or negative, while QtFIT results were quantitatively measured using the Respons® 910 (DiaSys Diagnostic Systems GmbH). Duplicate testing was performed to assess sample variability via the coefficient of variation (CV).
Agreement between QlFIT and QtFIT results was evaluated using Cohen's kappa (κ) statistic, and differences were assessed with the t-test ($\alpha = 0.05$).

Results: QtFIT detected 56.2% positive samples, with a predefined manufacturer cutoff of 100 µg/g for positivity, whereas QlFIT detected 40.6% positive results. The mean QtFIT result was 117.3 µg/g (95% confidence interval: 92.98–141.63), with a CV of 35.8% for duplicate measurements. κ statistic was 0.21, indicating slight agreement between the two methods. The t-test statistic (0.169) suggested no significant difference between QlFIT and QtFIT results.

Conclusion: The low κ showed limited agreement between QlFIT and QtFIT. However, the t-test results indicated no statistically significant difference between them.
Given the heterogeneous nature of stool samples, improved sampling techniques are required to minimize within-sample variability. Also, screening programs should select positivity thresholds based on the desired specificity and manageable positivity rates. Adjusting cutoff values may help standardize results and optimize screening efficiency.

P66

INFLUENCE OF THE ASSAY FOR MEASURING SERUM ALBUMIN

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Introduction: Serum albumin is an important prognostic biomarker and is used in the treatment of various clinical conditions. The gold standard for its measuring is nephelometry, a costly procedure. In practice, dye-binding methods are commonly used, especially bromocresol green (BCG), and less often, bromocresol purple (BCP). Immunoturbidimetry (IT) is another less frequently employed technique. Recent evidence indicates that serum albumin levels vary significantly based on the laboratory method used. Objective: This study aims to assess the magnitude of the discrepancy in serum albumin levels measured by BCG, BCP, and IT.

Methods: We measured serum albumin levels by BCG, BCP and IT in 60 serum samples divided into three groups: inflammatory pathology (IP), hemodialysis (HD) and control (CO).
Inclusion in the IP group required a C-reactive protein (CRP) level above 100 mg/dL.
The HD group included hemodialysis patients, while the CO group consisted of subjects with various medical conditions and normal CRP levels. Analyses were performed on Alinity c equipment, using Abbott® and DiAgam® reagents for dye-binding methods and IT, respectively. Statistical analyses were conducted with GraphPad Prism 5.03®, employing parametric (One-way ANOVA/ Bonferroni's test) or nonparametric (Friedman ANOVA/ Dunn's test) methods, depending on data distribution. Values were expressed as mean ± SD, and a p value < 0.05 was considered statistically significant.

Results: Serum albumin measurements in the CO group were 4.1 ± 0.6 g/dL (BCG), 3.8 ± 0.6 g/dL (BCP), and 3.7 ± 0.5 g/dL (IT).
In the IP group, albumin levels were 3.1 ± 0.7 g/dL (BCG), 2.5 ± 0.8 g/dL (BCP), and 2.5 ± 0.7 g/dL (IT). In both groups, serum albumin levels obtained by BCP were in good agreement with IT values, while BCG levels were significantly higher ($p < 0.05$).
In HD group, serum albumin means differed statistically ($p < 0.05$) among the three assays as follows: 3.5 ± 0.9 g/dL (BCG), 2.9 ± 1.0 g/dL (IT) and 2.7 ± 1.0 g/dL (BCP).

Conclusions: This study confirms a discrepancy among albumin assays, particularly a significant overestimation of albumin levels with the BCG method.
Overall, IT and BCP values show good concordance, except in hemodialysis patients, where a negative bias is observed in the BCP method. Given that variations in methods can lead to discrepancies in clinical decision-making, we highlight the need for better standardization of serum albumin measurements.

P67

HOW TO PERFORM THE LABORATORY DIAGNOSIS OF VITAMIN B12 DEFICIENCY?

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Introduction: The total Vitamin B12 (B12T) determination presents some limitations, since most of the measured cobalamin is bound to haptocorrin, so it is not available to most cells. Only 10-30% of Vitamin B12 is in the active form, Holotranscobalamin (B12A), that presents a shorter half-life, so it can be an early biomarker of deficiency, even before clinical manifestations.

Aim: The aim of this study was to compare the ability of two biomarkers (B12A and B12T) to identify deficiency and associate with hematological alterations.

Methods: We included patients with B12A and B12T simultaneous determination (1/5/2024 to 31/1/2025). Chemiluminescent microparticle assay was used to quantify B12A and B12T in serum (Alinity i).
The results interpretation was performed according to National Institute for Health and Care Excellence (NICE) guideline “Vitamin B12 deficiency in over 16s: diagnosis and management”.

The correlation between B12A and B12T was performed using Spearman test (GraphPad Prism 10).

Results and discussion: A moderate correlation was found between B12T and B12A.
Following the interpretation according to the reference values (suggested in the package inserts), in 95.3% of the determinations of both biomarkers deficiency was excluded. According to the NICE guideline interpretation deficiency is excluded in 63.3% of patients considering the B12A results and in 72.7% considering the B12T results. 32.0% of patients are classified as "Possible Vitamin B12 Deficiency" considering the B12A results and 23.2% considering the B12T results. These results may raise the hypothesis that a decrease in B12A is already observed in some patients with B12T values indicating that "Vitamin B12 deficiency is unlike".
Among the patients classified as "Possible vitamin B12 deficiency" considering the B12A results, 49.6% were classified as "Vitamin B12 deficiency is unlike" considering the B12T results and among them 6.8% presented alterations compatible with macrocytic anemia.

Conclusion: These results appear to confirm that B12A may be an earlier biomarker and has a greater ability to identify deficiency.
To reinforce these conclusions, it would be essential to determine homocysteine in patients in whom it was not possible to confirm or exclude deficiency and assess other clinical manifestations, or perform other determinations in these patients. These preliminary results are part of a more comprehensive project to define an algorithm to evaluate vitamin B12 deficiency.

P68

SIMULTANEOUS QUANTIFICATION OF AZATHIOPRINE METABOLITES IN ERYTHROCYTES BY TANDEM MASS CHROMATOGRAPHY: NECESSITY OR FAD?

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Introduction: Azathioprine (AZT) is an immunosuppressive drug used in the treatment of inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus and other autoimmune diseases. AZT is a prodrug of 6-mercaptopurine (6MP), a purine antagonist. 6MP requires cellular uptake and intracellular metabolism to thioguanine (TG) nucleotides (TGN) and 6-methyl-mercaptopurine (6MMP), inhibiting purine synthesis and purine nucleotide interconversions. The treatments may be associated with side effect, mainly myelosuppression and hepatotoxicity. Patients with deficient activity of TPMT or with the NUDT15 c.415C>T gene mutation reveal an increased risk of severe toxicity at conventional doses, requiring rigorous and regular monitoring. Therefore, the quantification of AZT metabolites (TGN and 6MMP) in blood is fundamental in monitoring the efficacy and safety associated with the azathioprine dosing regimen.

Aim: The authors present a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for simultaneous quantification of 6MMP and TGN in erythrocytes (RBC).

Methods: The LC-MS/MS assay was established according to Lie Li et al (2024), where 6TG is representative for TGN.
Patients EDTA samples were collected after steady state and washed with Hanks' Balanced Salt Solution (HBSS) (Sigma-Aldrich®) before being frozen at -80ºC for, at least, 24h. Some patients were clinically stable, others were not.
Internal standards were purchased from LGC® and calibrators from Sigma-Aldrich®, using curves ranging from 0.819–200 nmol/mL for 6MMP and 0.102–25 nmol/mL for 6TG. An Atlantis T3 3µm 2.1x100 mm column (Waters®) was used. The 2 specific m/z transitions were: 168>134/168>151 and 158>55/158>110, for 6TG and 6MMP, respectively.

Results: The calibration curves exhibited outstanding linearity (r^2>0.995) for both nucleotides. Imprecision (CV%) was <10%. The 6TG and 6MMP results were normalized to 4x1012 RBC/L and a good agreement was found between the clinical condition and the quantified concentrations but not between dose and AZT metabolites quantified.

Conclusion: This LC-MS/MS method has demonstrated specificity, accuracy, and sensitivity for quantifying AZT metabolites in erythrocytes (6MMP and 6TG), being well-suited for integration into laboratory workflow. The results are highly encouraging of the need for AZT therapeutic monitoring, given the high variability between concentrations and doses, preventing inefficacy and adverse effects.

P69

LIPID PROFILE CHARACTERIZATION IN AN OCCUPATIONAL MEDICINE COHORT

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The lipid profile serves as a critical indicator for assessing cardiometabolic risks, particularly in working populations where occupational stress, sedentary behaviour, and unhealthy dietary habits can adversely impact cardiovascular health. This study aimed to characterize the lipid profiles of an adult cohort assessed within the framework of occupational medicine, providing data to inform prevention and health promotion strategies in workplace settings.

A total of 706 adult workers from diverse occupational sectors participated in this study. Participants underwent routine medical evaluations as part of occupational health assessments. Fasting blood samples were collected to measure lipid parameters, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides. The data were analysed to determine the prevalence of dyslipidaemia, with stratification by age, sex, diabetes status and high blood pressure (HBP). Statistical analyses were performed using descriptive and inferential methods with IBM SPSS Statistics v.29.0 (IBM Corp., Armonk, NY). A p-value of < 0.05 was considered statistically significant for all inferential analyses.

Our findings revealed that 72.9% of participants exhibited elevated LDL-C, and 50.5% had increased TC.

Conversely, 80.9% and 62.9% of the cohort demonstrated ideal triglyceride andHDL-C levels, respectively. The prevalence of diabetes and HBP was 10.6% and 23.5%, respectively. Elevated LDL-C levels were significantly associated with male participants (p= 0.016), and dyslipidaemia prevalence was notably higher among workers aged over 40. Our results align with prior studies by Chora et al. (2024) and Gavina et al. (2022), which reported that while the lipid profile of the Portuguese population generally falls within recommended ranges, a substantial proportion (over 50%) exhibit lipid parameters exceeding recommended levels, particularly for TC and LDL-C.

Regular monitoring of lipid profiles in occupational health programs is essential for the early identification of cardiovascular risk factors. Furthermore, targeted workplace interventions, such as promoting healthier lifestyles, can significantly enhance the overall health of workers.

Our study underscores the importance of integrating public health measures into occupational health frameworks, offering valuable insights to guide the development of more effective workplace health policies.

P70

EVALUATING EGFR: A COMPARISON OF CKD-EPI AND EKFC EQUATIONS

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Introduction: The glomerular filtration rate (GFR) reflects the rate at which blood is filtered by the glomeruli per unit of time.

Estimated glomerular filtration rate (eGFR) serves as a crucial parameter in the diagnosis and classification of chronic kidney disease (CKD). eGFR can be measured by various formulas, with the most commonly used being Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) or European Kidney Function Consortium (EKFC) equations. These formulas estimate GFR based on serum creatinine levels, age and gender.

Objective: Compare eGFR results obtained from CKD-EPI2021 and EKFC equations on an oncologic hospital.

Materials and Methods: A total of 48737 serum samples from whole 2023 year were evaluated. Creatinine was measured on Atellica CH system by Siemens Healthineers®, with a Limit of Detection (LoD) ≤ 0,10 mg/dL. Limits of jaundice, haemolysis and lipemia were respected. EGFR equations used were CKD-EPI2021 and EKFC. Non-parametric Wilcoxon test was performed on SPSS.

Results: The age of patients ranges from 18 to 103 years (mean age 64.8), with 61.11% women and 38.89% men. Mean value of eGFR with CKD-EPI2021 was 85.31 mL/min/1.73m2 (deviation 22.14). Mean value of eGFR with EKFC was 75.84 mL/min/1.73m2 (deviation 21.18). The EKFC values were lower than CKD-EPI2021 (48638 times on 48737 total). A Wilcoxon test confirmed significant differences between both equations results (p ≤ 0.05).

Discussion/Conclusion: The mean values of eGFR are below the normal range of glomerular filtration (> 90 mL/min/1.73m2) on both models, corresponding to a G2 category of GFR.

This is expected, since the study population has oncologic pathology and/ or other comorbidities associated. Although CKD-EPI2021 has good results for general population and near normal GFR, it was developed with an American population. In opposite, EKFC was developed using a European population, with a Q value determined according to the specific population where it is applied. EKFC is better adjusted to specific populations such as ours. It seems recommendable EKFC equation use and report, although more studies are needed, especially ones where Q value for the study population is determined, in order to provide most accurate results. The most adjusted equation for the country and population in study should be used, so, studies within Portuguese population and different regions of the country are needed.

P71

24-HOUR URINE IMMUNOFIXATION IN AL AMYLOIDOSIS DIAGNOSIS: LIKELY THE ONLY CONTEXT WHERE IT REMAINS ESSENTIAL.

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Recent studies have shown that analysis of 24-hour urine samples does not provide additional information compared to serum analysis in the study of monoclonal protein (MP) in plasma cell dyscrasias if serum protein electrophoresis (SPE), serum free light chains (FLC) and serum immunofixation (IFS) are combined. The development of FLC assay has enabled the detection of FLC at low concentrations with high sensitivity, rendering the 24-hour urine test largely dispensable. However, exceptions may exist for patients with AL Amyloidosis.

From 2016 to 2024, we reviewed a group of 15 patients diagnosed with AL amyloidosis, along with one patient still under investigation to confirm the diagnosis. Among them, 6 had intact immunoglobulin (3 IgG Lambda, 3 IgA Lambda), while 10 had Lambda light chain only. The 24-hour urine immunofixation (IFU) (by gel electrophoresis, Hydrasys®, Sebia) was positive for all patients. IFS (by gel electrophoresis, Hydrasys®, Sebia) was positive in 10 patients, of which 6 had measurable MP in SPE (by capillarys, Octa®, Sebia).
The free light chain ratio (rFLC) (by Freelite® assay) was abnormal in 15 patients. The 6 patients that had a negative IFS were all light chain only; one of these patients was only positive in IFU and had a normal rFLC.

In our study of AL amyloidosis patients, we observed that all presented with a Lambda isotype, which is consistent with the typical presentation of this disease. In some cases, due to the nature of AL amyloidosis, in which the light chains tend to misfold and aggregate into amyloid deposits in various tissues and organs, only a small amount of MP is detected in the patients' serum. The FLC assay proved to be crucial, showing abnormal rFLC in 15 out of 16 patients.

This assay not only aids in diagnosis but also serves as an important prognostic marker during follow-up. Interestingly, only one test, the IFU, was positive across all patients, and in one case, it was the exclusive method for identifying MP.

Despite the advancements in diagnostic techniques, immunofixation remains the gold standard for the characterization of monoclonal immunoglobulins.
While the 24-hour urine test may not be necessary for diagnosing most plasma cell dyscrasias, it still holds significant value in the diagnosis of amyloidosis. Each test provides unique insights, and when combined, they ensure that no critical information is missed.

P72

IGD MULTIPLE MYELOMA: FEATURES OF ITS DIAGNOSIS

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Introduction: Multiple myeloma (MM) is typically characterized by the proliferation of plasma cells that produce high levels of a monoclonal protein. It accounts for approximately 1-2% of neoplasms, and 17% of hematological neoplasms. The most common subtype is IgG, with IgD being a rare subtype, corresponding to 1% of MM cases. The diagnosis is made by the presence of two criteria: at least 10% clonal plasma cells in the bone marrow, or presence of extramedullary plasmacytoma, and any of the following - target organ damage: presence of hypercalcemia, renal failure, anemia and bone lesions (CRAB); >60% clonal plasma cells in the BM; light chain ratio >100; >1 focal lesion on MRI.
We present a case of MM IgD kappa, which, due to its rarity, we thought it pertinent to report.

Case description: A 65-year-old woman with a history of systemic lupus erythematosus, hypertension and morbid obesity was referred to neurosurgery for a lytic lesion in C5.
There was no evidence of cytopenias, calcium alterations or immunoglobulin, and free light chain assays were normal.
The immunofixation initially showed a slight band in the reference band, with no bands identified in the other lanes. Therefore, at the initiative of the clinical pathologist, a new immunofixation was carried out to study the IgD and IgE chains, and an IgD kappa monoclonal band was identified.
The peripheral blood smear showed some erythrocyte “rouleaux” and the myelogram showed 8% plasma cells and 3% plasmablasts. Immunophenotyping of the bone marrow revealed the presence of 0.2% plasma cells, 16% of which were kappa clones.
The patient underwent corpectomy and placement of a cylinder and plate and treated with urgent radiotherapy and then started on VTD (bortezomib + thalidomide + dexamethasone).

Discussion: Multiple myeloma is a relatively rare disease, accounting for 1-2% of neoplasms, and the IgD subtype is even less frequent, accounting for 1% of MM. This case highlights the role of the clinical pathologist in diagnosing this rarer subtype of MM, with a less typical presentation and no initial laboratory alterations suggesting this disease.

P73

HEAVY/LIGHT CHAIN: A BIOMARKER THAT CAN PREDICT DISEASE STATUS DURING THE FOLLOW-UP OF INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA

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Despite all the innovations in therapies in recent years, multiple myeloma (MM) remains incurable. Disease progression during treatment or short remission periods require more frequent monitoring, allowing quicker and effective clinical decisions. Clinicians should have access to biomarkers that help to identify patients at greater risk of relapse.
The Hevylite® assay allows simple and automated quantification of specific heavy chain/light chain (HLC) pairs of immunoglobulins. After monoclonal isotype recognition, it is recommended, to test both pairs of the monoclonal isotype.
We retrospectively selected 10 patients with MM of the IgA subtype, who were undergoing treatment, and in a possible change in disease status.
At least 2 assessment points were considered for: total IgA measurements (cobas® 8000, Roche), IgAk and IgAλ (Hevylite®), and monoclonal protein (MP) in Capilar Zone Eletrophoresis (CZE) (minicap®, Sebia). Free light chains (FLC) were also evaluated (Freelite®).

The results show that HLC provides information on the presence of MP at more assessment points than CZE. When Identifying MP using these methods, HLC is more sensitive and allows for quantification. The information from total IgA is easily surpassed by the discrimination of HLC into IgAk and IgAλ. In some patients, total IgA, although within the considered normal range, is entirely pathological due to immunosuppression of the uninvolved HLC.
The detailed analysis of 8/10 patients, suggest that HLC might consistently anticipate changes in disease status compared to CZE. These are patients in whom progression during treatment has been confirmed. The migration site of the MP in CZE, specifically in Beta 1 zone, or at multiple locations in the CZE trace, puts CZE at a disadvantage in identifying MP.

The sFLC measurements in 1 patient was normal, which makes HLC a biomarker with greater relevance in the early detection of MP.
This pilot study anticipates the significant role that HLC may have in early detection of changes in disease status in patients with intact immunoglobulin MM undergoing treatment.
It is essential to extend this study to include patients with other Ig isotypes throughout the entire follow-up period, to define the essential points for HLC evaluation, and to refine the minimum value changes with clinical significance.

P74

SYSTEMIC SCLEROSIS AND PRIMARY BILIARY CHOLANGITIS: CLINICAL AND SEROLOGICAL INSIGHTS

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Introduction: Systemic sclerosis (SSc) and primary biliary cholangitis (PBC) are autoimmune diseases that can coexist, suggesting a potential immunological link. PBC is the most common autoimmune liver disease in SSc, particularly in its cutaneous form (SSc).
The presence of anti-centromere antibodies (ACA) in SSc and anti-mitochondrial antibodies (AMA) in PBC raises questions about their clinical significance. This study aims to assess the clinical and serological characteristics of patients with both SSc and PBC, evaluating autoantibody prevalence and potential prognostic implications.

Materials and Methods: A cohort study was conducted over four years (2020–2024) at a tertiary hospital, analyzing the clinical and laboratory data of 162 patients.
The detection of autoantibodies involved multiple methods, including indirect immunofluorescence for antinuclear antibodies (ANA) and AMA on HEp-2 cells. In addition, immunoblot assays were used to identify autoantibodies specific to SSc and autoimmune liver diseases, including Gp210, Sp100, CENP-B, and CENP-A. Enzyme-linked immunosorbent assay (ELISA) was utilized to detect anti-mitochondrial M2 antibodies (AMA-M2).

Results: Of the 162 patients analyzed, 25% (41 patients) tested positive for AMA, which is associated with PBC, at the time of diagnosis.
Notably, 45% of newly diagnosed patients (18 patients) showed the presence of both ACA and AMA, suggesting a potential immunological overlap between SSc and PBC. The median age of the cohort was 57 years, with a predominance of female patients (86% women, 14% men).
Clinically, 14% of patients (23 individuals) exhibited symptoms consistent with both SSc and PBC at the time of diagnosis, including Raynaud's phenomenon, telangiectasias, sclerodactyly, esophageal dysmotility, and cholestatic liver dysfunction. The remaining 86% (139 patients) were seropositive for ACA and/or AMA but were asymptomatic at the time of diagnosis.

Conclusion: This study highlights the clinical and serological overlap between SSc and PBC, suggesting that the presence of ACA and AMA could influence disease progression and prognosis. It underscores the importance of routine screening, long-term monitoring, and early detection for effective management of these patients.

P75

TRACE ELEMENT LEVELS AND THEIR ASSOCIATION WITH BIOCHEMICAL PARAMETERS IN HEMODIALYSIS PATIENTS

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Introduction: Renal patients undergoing haemodialysis (HD) experience biochemical, haematological and inflammatory alterations due to impaired kidney function and dialysis-related factors. These include anaemia, disturbances in creatinine, urea, mineral metabolism and inflammatory markers like ferritin. Trace elements (TE) imbalances are also, frequently, observed potentially influencing oxidative stress, the immune system and metabolism.

Objectives: To analyse serum TE levels in HD compared to healthy individuals (control group [CT]), and their associations with biochemical and haematological parameters.

Methods: A total of 126 HD patients and 38 CT were included in this study. Haematological (red blood cells [RBC], haematocrit, [Hct] mean corpuscular haemoglobin [MCH)]) and biochemical parameters (urea, creatinine, albumin, calcium, phosphorus, iron and ferritin) were assessed. Serum macro-and TE levels were analysed by inductively coupled plasma–mass spectrometry. Spearman's rank correlation test was used to study the existence of correlations between the analysed variables.

Results: HD patients had significantly lower RBC count and Hct, while MCH remained normal. Urea, creatinine, and ferritin levels were significantly elevated ($p<0.001$), whereas albumin, calcium, phosphorus, and iron were within reference ranges. Correlational analysis indicated positive associations between barium and albumin ($\rho=0.227$, $p=0.030$), lithium and ferritin ($\rho=0.255$, $p=0.015$), magnesium and urea ($\rho=0.290$, $p=0.005$), cobalt and phosphorus ($\rho=0.287$, $p=0.006$), cadmium and iron ($\rho=0.261$, $p=0.026$), and cadmium and haematocrit ($\rho=0.245$, $p=0.019$). Negative correlations were found between MCH and magnesium ($\rho=-0.323$, $p=0.002$), zinc ($\rho=-0.280$, $p=0.007$), and selenium ($\rho=-0.229$, $p=0.028$).

Conclusion: These results confirm the expected haematological and biochemical changes in HD, as well as changes in TE. It also reveals that TE imbalances are related to biochemical, haematological and inflammatory changes. Further research is needed to clarify the clinical significance of these associations and their potential implications for the treatment of patients.

P76

METHOTREXATE AND ITS TOXICITY

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Introduction: Methotrexate (MTX) is a structural analogue of folic acid with antiproliferative and immunomodulatory properties, used in the treatment of oncological and autoimmune diseases. In high doses, it is generally used in the treatment of hemato-oncological pathology. The toxic effects are well known, especially in patients with hypoalbuminemia, renal failure, folate deficiency or polymedication. Genetic polymorphisms may increase susceptibility to MTX.

Clinical Case: A 60-year-old man, referred due to persistent neck pain, presented, on magnetic resonance imaging, an extra-medullary cervical lesion. After laminectomy, he was diagnosed with B-lymphoid neoplasia, classified as Diffuse Large B-Cell Lymphoma (DLBCL). History of arterial hypertension, prostatic hypertrophy and laparoscopic cholecystectomy. Usual medication: pantoprazole, cotrimoxazole, acyclovir, allopurinol, alfuzosin, sertraline and alprazolam. It was decided to start the R-CHOP chemotherapy protocol, associated with MTX for central nervous system prophylaxis. A single dose of MTX was administered, at 86% of the protocol dose, totaling 3 g/m2. Laboratorial monitoring was performed, measuring MTX blood levels using the "Enzyme-Multiplied Immunoassay Technique" (EMIT®) method on the VIVA-E® equipment (Siemens Healthineers®), as well as parameters such as creatinine, liver enzymes and albumin. Prior to MTX, the patient had normal creatinine levels (1.0 mg/mL), with near normal (86.16 ml/min/1.73m2) estimated glomerular filtration rate (eGFR).

24 hours after MTX administration, creatinine was 2 mg/mL, with eGFR 37.50 ml/min/1.73m2, indicating grade G3b renal failure. Slightly altered liver function with slight increase in transaminases. MTX elimination was abnormally slow, reaching the target value of 0.1 µmol/L only 144 hours after MTX administration, with eGFR low throughout approximately 2 months.

Conclusion: This case demonstrates how crucial it is, when faced with a diagnosis of DLBCL, the importance of pharmacokinetics evaluation and laboratorial monitoring to optimize treatment and assess potential undesirable effects. Carrying out a prior pharmacogenomic study and pharmacological consultation would be beneficial, due to possible interactions between methotrexate and the patient's usual medication. Although MTX is a valid therapeutic, a more personalized approach, with laboratory support, can avoid treatment interruptions and adverse effects as the one described.

P77

LABORATORY CHALLENGES: IMMUNOGLOBULIN QUANTIFICATION IN A PATIENT WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is a malignant monoclonal gammopathy, characterized by the excessive production of monoclonal immunoglobulins (Ig). Accurate quantification of monoclonal Ig in these patients can be difficult, with factors such as the quantity and specific properties of the Ig interfering with the laboratory methods used for its quantification.

A 63-year-old male was admitted to the emergency department following a traffic accident. Initial evaluation revealed anemia (hemoglobin: 6.5 g/dL), rouleaux formation, acute kidney injury, hyperproteinemia (total protein (TP): 12.5 g/dL), and hyperproteinuria (255.5 mg/dL).
Regarding these results the patient was directed to the Clinical Hematology Department with suspicion of MM. During laboratory workup, after centrifugation, the sample revealed an atypical distribution of the separating gel. The centrifugation time was prolonged, followed by heating in an incubator at 37°C. The serum had a gelatinous consistency, but we were able to obtain some serum to perform the prescribed laboratorial analysis. By an Immunoturbidimetric assay (Cobas® 8000, Roche Diagnostics), the IgA levels were significantly elevated, 6772 mg/dL. Electrophoresis was performed, revealing a monoclonal protein (MP) of 7400 mg/dL in the γ zone, identified by Immunotyping as an IgA/κ.

This case aims to highlight the challenges in processing samples with high Ig levels.

Since the volume of the first sample was insufficient, we used a second and third samples obtained after the beginning of the MM treatment.
The quantification of monoclonal IgA was performed with kit Hevylite® (Optilite, Binding Site) that provides accurate quantification of IgA by measuring the heavy and light chain pairs of Ig (IgAκ and IgAλ). In the 2nd sample, the total IgA level was 6320 mg/dL, the MP in the electrophoresis was 7000 mg/dL and the IgAκ was 7864 mg/dL.

During MM treatment, a decrease in MP is expected as an indicator of therapeutic response. This was not immediately observed with total IgA, but was evident in the free light chains and TP. One month later, a decrease in IgA levels was noticeable (total IgA: 3725 mg/dL, MP: 3800 mg/dL and IgAκ: 5817 mg/dL).

This study showed that the IgAκ was higher than total IgA, leading to our initial suspicion of under-quantification of total IgA. This may be attributed not only to poor serum separation due to elevated MP levels, which led to limited serum volume, but also to the equipment's quantification limits.

P78

STATINS AND AUTOIMMUNITY: PROFILING PATIENTS WITH ANTI-HMGCR MYOPATHY

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Introduction: Statins are known to be an effective lipid lowering drugs commonly prescribed to prevent cardiovascular diseases. Although their benefits, statins may have several side effects related to muscle toxicity.
A rare complication of statins is the immune-mediated necrotizing myopathy (IMNM), a muscle disorder characterized by inflammation and necrosis of muscle fibers, frequently associated with the presence of autoantibodies against anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (anti-HMGCR). By competitively inhibiting HMGCR, statins lead to a compensatory increase of this enzyme in muscles. This increment raises the probability of antigen-presenting-cells abnormally process HMGCR, triggering an autoimmune process. Diagnostic criteria for IMNM includes proximal muscle weakness, markedly elevated serum creatine kinase (CK), myopathic electromyography and muscle biopsies consistent with necrotizing myopathy. Long-term immunosuppressive therapy is required for this disease.

Objective: To characterize the population of a central hospital with anti-HMGCR positivity and assess the association between statin use and the development of IMNM.

Methods: A retrospective analysis was conducted using data from sample analysis that included the immunodot assay BlueDot Myositis DOT Kit (D-tek®). Patients with positive anti-HMGCR were selected.

Results: In the last 6 years, a total of 158 immunodot assays were required by the clinicians when autoimmune myopathy was suspected. Approximately 4.4% (7 samples) were positive to anti-HMGCR; 43% were female. The median age was 68 years-old (59 - 77). 6 samples (85.7%) were exposed to atorvastatin and 1 had no information on statin exposure. All of them presented proximal bilateral muscle weakness (57% on thighs and 43% on thighs and shoulders). At the first attending to the doctor, serum CK levels were 14 to 67 times higher than the reference range (< 145 U/L). Transaminases were above the reference range. There were no B12 vitamin deficits or changes in thyroid and renal function.

Conclusions: Although HMGCR-associated IMNM is a rare form of myositis, it should be considered as a potential cause of proximal muscle weakness and persistent serum CK elevation in patients exposed to statins. This reinforces the need for continuous monitoring of these patients to ensure early diagnosis and timely therapy.

P79

DE RITIS RATIO AND CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) IN THE ASSESSMENT OF HEPATIC INJURY: SOMETHING OLD SOMETHING NEW

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Introduction: The De Ritis ratio (aspartate transaminase-AST/alanine transaminase-ALT ratio) is a laboratory determination mainly recognized in diagnosis and management of liver diseases, being also associated with other conditions, such as cardiovascular disorders and muscular pathologies, thus having an important prognostic value.
In the liver disease approach, it has been often used to distinguish between alcoholic hepatitis and nonalcoholic steatohepatitis (NASH), with a value greater than 2:1 related to severe alcoholic disease and fibrosis, and less than 1:1 related to NASH.
Carbohydrate-deficient transferrin (CDT) can occur due to congenital diseases, acquired hepatic impairments and heavy alcohol consumption, being used as an abuse biomarker.
Since the two parameters are used in alcoholic liver disease, the authors formulated an assessment of their complementarity.

Objective: Assess the magnitude of correlation between CDT measurements and AST to ALT ratio determinations in a population with liver disfunction.

Methods: A one-year retrospective observational analysis of the results of CDT petitions, in the context of liver disease, and the respective AST/ALT ratio was carried out.
Population was divided in two groups: pediatric and adult. AST and ALT results were obtained using Alinity® c (Abbott®). CDT was obtained with MINICAP® (Sebia®).
A cut-off of 2:1 was used for the AST/ALT ratio and >1.7% for %CDT. Data was statistically analysed using Microsoft Excel®. Complementarity was searched using Person's correlation.

Results: A total of 247 patients were analysed, 138 of which pediatric patients (56%). In the pediatric population (<18 years) it was assumed that no alcohol consumption existed. 35 had an AST/ALT ratio > 2 and 18 CDT > 1.7%. The correlation between these results was very weak and negative (r=-0.186). In adults, 20 had an AST/ALT ratio > 2 and 29 CDT > 1.7%. No linear relationship was found between the results (r=0.027) but when only the AST/ALT ratio > 2 is considered, a weak negative correlation exists with the CDT values (r=-0.239).

Conclusions: Correlations between the pediatric and adult populations with a ratio >2.0 are identical, suggesting a weak relationship in the studied population between liver disease and severe alcohol consumption. Additional prospective studies will be necessary to clarify the value of combined biomarkers in the assessment of hepatic disease.

P80

EVALUATING TECLISTAMAB EFFICACY IN MULTIPLE MYELOMA: THE CRITICAL ROLE OF FREE LIGHT CHAIN BIOMARKER. RESULTS FROM A SMALL COHORT OF PATIENT

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Teclistamab, a bispecific antibody targeting CD3 on T-cells and B-cell maturation antigen on plasma cells, has shown significant potential in treating relapsed or refractory multiple myeloma (RRMM). The Free Light Chain (FLC) biomarker plays a crucial role in diagnosis and monitoring of patients with multiple Myeloma (MM). Regular assessment of FLC levels is essential for evaluating the effectiveness of the treatment by monitoring the decrease in the production of pathogenic FLC, which are markers for disease progression but also responsible for potential organ damage.

A small cohort of RRMM patients undergoing treatment with Teclistamab in our center included the following MM subtypes: 3 IgA Kappa, 2 IgG Kappa, 1 IgG Lambda and 1 Lambda light chain only. All patients had an abnormal FLC ratio at the start of treatment.
At the first evaluation point after treatment initiation: 1 patient (IgG Kappa) stopped treatment due to neurologic toxicity; 4 patients had FLC levels below the limit of the test quantification (kappa free (KF) <0.03; Lambda Free (LF) < 0.07, mg/dL); 1 IgG Lambda patient had KF<0.03 and LF=0.32, mg/dL; 1 IgG Kappa patient had only the initial evaluation point (KF= 101.26; LF<0.07, mg/dL).

In the group of 4 patients with FLC levels below the limit of the test quantification, 3 are in complete response (CR) and have been followed for 10, 13 and 14 months.
The 4th patient (IgA Kappa) was also in CR but, unfortunately, died due to infection complications. The IgG Lambda patient eventually achieved a good response in FLC assessment at the 3rd evaluation point (KF<0.03; LF< 0.07, mg/dL), however, at the 4th assessment, a small increase in LF (KF<0.03; LF= 0.20, mg/dL) raised the suspicion of disease progression which was later confirmed.

The results seem to indicate that:

1. A significant reduction in FLC levels at the 1st evaluation point might correlate with a significant hematologic response and achievement of CR.
2. A slight increase in the involved FLC concentration may be sufficient to identify disease progression.
3. Despite the limited number of patients, these results indicate that when assessing the efficacy of Teclistamab treatment in MM, FLC may be a crucial marker for patient monitoring due to its sensitivity and quick response to changes in disease state.

P81

TECLISTAMAB – THE ASSESSMENT OF INTERFERENCES ON LABORATORIAL RESULTS IN MULTIPLE MYELOMA PATIENTS

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Multiple myeloma (MM) represents 1% of all cancers and about 10% of hematologic malignancies. Despite treatment advances in recent years, MM remains an incurable disease.
According to IMWG criteria, serum protein electrophoresis (SPE) and serum immunofixation (IFE) are essential for diagnosing, risk stratifying and assessing treatment response in MM patients.
Teclistamab (Tecvayli™) is a recent approved bispecific IgG Lambda antibody and the first of its class for treating refractory MM. It is a humanized IgG4 antibody that features one arm targeting B-cell maturation antigen (BCMA) and CD3 receptors. By binding to both BCMA on myeloma cells and CD3 on T cells, teclistamab effectively recruits and activates T cells to destroy myeloma cells.
Published studies have demonstrated that therapeutic monoclonal antibodies used to treat MM are detectable by SPE and IFE, which can interfere with accurate evaluation of complete response. In this study, we evaluated the interference of teclistamab therapy on SPE trace and IFE results.
We start by performing IFE (Hydrasys®, Sebia) using teclistamab as a sample in order to determine its migration zone. The result showed an IgG Lambda at the end of gamma region.
Four MM patients undergoing teclistamab (10 mg/mL) treatment were selected. A total of 40 SPE (Capillarys 3 Octa®, Sebia) and 15 IFE (Hydrasys®, Sebia) were performed to assess the complete response to teclistamab. The first sample evaluated from each patient correspond to the assessment at the start of treatment and the remaing samples to follow up. In the patients evaluated, the immunoglobulin isotype was different from that of teclistamab, 1 light chain lambda, 1 IgA kappa and 2 IgG kappa. The migration zone of the monoclonal protein differed from Teclistamab migration zone, which further facilitated interpretation, as there was no possibility of co-migration in the same zone. In all samples, SPE and IFE analysis did not reveal IgG lambda on Teclistamab migration zone.

This study demonstrates that treatment with this therapeutic bispecific IgG Lambda antibody does not interfere with the electrophoresis trace or the immunofixation results. However, it is important to expand this study to include a larger patient population to better understanding the analytical interference of this drug.

P82

EVALUATION OF THE USEFULNESS OF ACTIVE VITAMIN B12 MEASUREMENT

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Introduction: The objective of this study was to evaluate the effectiveness of active vitamin B12 measurement compared to the total vitamin B12 test.
Vitamin B12 is an essential nutrient for neurological and hematopoietic function, and its deficiency can result in serious conditions such as megaloblastic anemia and neuropathy.

Methodology: Over a period of 6 months, 2117 total vitamin B12 tests were performed using a chemiluminescent microparticle immunoassay on the Abbott Alinity i analyzer, with the B12 Reagent Kit (reference values: 187 - 883 pg/ml).
For the 448 sera with total vitamin B12 results in the gray zone (150-300 pg/ml), active vitamin B12 measurements were performed using the same analyzer with the Active_B12 (Holotranscobalamin) Reagent Kit (reference values: 25.1 - 165 pmol/l).

Results: Of the 448 sera in the gray zone of total vitamin B12:
171 had active vitamin B12 >70 pmol/l
254 had active vitamin B12 between 25 and 70 pmol/l
23 had active vitamin B12 <25 pmol/l

Discussion: The results indicate that active vitamin B12 measurement can provide a more precise assessment of vitamin B12 levels, especially in cases where total vitamin B12 results are inconclusive.
Indeed, it was observed that 23 patients with normal total vitamin B12 levels were found to have a deficiency when assessed by active vitamin B12, highlighting the importance of including this test in the diagnostic protocol. Additionally, it is important to note that the reference range for total vitamin B12 is very broad (187 - 883 pg/ml), which can lead to difficulties in interpreting the results and necessitates complementary tests for a more accurate assessment.
Comparative analysis suggests that active vitamin B12 measurement can reduce false positives and negatives, thereby improving diagnostic accuracy and treatment effectiveness.

Conclusion: Integrating the active vitamin B12 test as a complement to total vitamin B12 measurement is recommended for a more accurate assessment of vitamin B12 levels. This is particularly important for patients with inconclusive results, allowing for a more reliable diagnosis and appropriate treatment.

P83

THE ROLE OF LEWIS BLOOD GROUP IN THE ASSESSMENT OF CA19.9

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Introduction: Carbohydrate antigen 19.9 (CA19.9) is a monosialylated Lewis (Le) blood group epitope (sialyl-LewisA) used to monitor bilio-pancreatic neoplasms and other malignant conditions. Its expression depends on the Lewis (Le) blood group phenotype. Individuals with a negative Lewis phenotype {Le(a-b-)} may present undetectable levels of CA19.9, even with diagnosed pathology, influencing their follow-up and monitoring.

Objectives: Evaluate the correlation between serum CA19.9 levels and the different Lewis blood group phenotypes, correlating this relationship with its clinical applicability.

Materials and methods: Serum and whole blood from 220 samples (91 with [CA19.9] <2.0 U/mL, 33 with [CA19.9] of 2–5 U/mL, and 96 with [CA19.9] of 5–10 U/mL) were used to quantify [CA19.9] and phenotypically characterize Le group, respectively.
[CA19.9] was measured using an electrochemiluminescence assay, Cobas pro e801 from Roche Diagnostics®, with a LoD of 2 U/mL. Le phenotype was determined by a haemagglutination reaction using specific monoclonal antibodies against Lea and Leb antigens (Immunocor).

Results: Lewis phenotypes for the study population were: 63.6% Le(a-b-), 35.5% Le(a-b+) and 0.9% Le(a+b-). The group with [CA19.9] <2.0 U/mL presented 100% Le(a-b-), while [CA19.9] of 2-5 U/mL presented 54.5% Le(a-b+) and 45.5% Le(a-b-) and [CA19.9] of 5-10 U/mL presented 63.5% Le(a-b+), 2.1% Le(a+b-) and 34.4% Le(a-b-).

Discussion/Conclusion: Results showed an association between Le(a−b−) phenotype and undetectable CA19.9 values.
The absence of CA19.9 release or synthesis in 100% of the individuals with Le(a−b−) phenotype was the expected. In the groups with [CA19.9] between 2-5 U/mL and 5-10 U/mL, there was a higher proportion of individuals with the Le(a−b+) phenotype.
It seems that, Le(a−b−) phenotype will, therefore, be a relevant factor to take in consideration when evaluating patients suspected of having bilio-pancreatic neoplasms.
However, in the group with [CA19.9] of 2-5 U/mL there is a large percentage of individuals with Le(a−b−), this may be explained by the proximity of assay limit of detection. Nevertheless, additional studies are needed to better understand the relationship between Le and [CA19.9], namely in a higher number of cancer individuals and individuals with increased [CA19.9], to confirm the results obtained.

P84

VARIABILITY IN TROPONIN LEVELS BY AGE AND GENDER: A COMPARATIVE ANALYSIS

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Troponins are proteins found in both skeletal and cardiac muscles involved in the regulation of muscle contraction. There are three main subtypes of troponin that make up the troponin complex: troponin C (cTnC), troponin I (cTnI) and troponin T (cTnT). cTnI is widely used due to its high specificity and sensitivity for the diagnosis of myocardial injury, being one of the main diagnostic and prognostic markers in cases of cardiovascular diseases, especially myocardial infarction and heart failure. However, its values appear to differ according to the age and sex of the individual. Therefore, the aim of our study was to evaluate whether there is variation in cTnI values with age and gender (men vs women), by chemiluminescent microparticle immunoassays (CMIA) in serum samples. We conducted a parameterized search between 2018 and 2024 at the Hospital in Coimbra and divided the participants into 8 groups: women under 47 years (n=130), women aged 48-63 years (n=308), women aged 64-80 years (n=725), and women over 81 years (n=982), men under 47 years (n=312), men aged 48-63 years (n=779), men aged 64-80 years (n=890) and men over 81 years (n=771).

Statistical Analysis used was: normality and variance homogeneity of the data were assessed using Shapiro-Wilk and Levene's tests, respectively. Significant differences identified by ANOVA were further analyzed with Tukey's HSD test, while Dunn's test was used for post hoc comparisons following the Kruskal-Wallis test. P-values were adjusted for multiple testing using the Benjamini–Hochberg method. We found that females (mean=8667) have lower mean cTnI values compared to males (mean=13959), with $p<0.05$, and that these values increase with age in females, possibly due to hormonal changes.
Women under 47 years of age(mean=6944) have lower mean cTnI values than men of the same age (mean=17895), with $p<0.001$. Women of before menopause have higher levels of estrogen, a hormone that offers some cardiovascular protection. This protective effect may mean that younger women have a lower risk of significant heart disease than men of the same age.
Women tend to develop heart disease later in life compared to men. After menopause, with the decrease in estrogen levels, women become more susceptible to cardiovascular problems, which may affect troponin levels.

Our study is in line with previous research and shows that cTnI is a good biomarker for heart disease, demonstrating differences between gender and age.

P85

LIPOPROTEIN A AND ITS IMPORTANCE IN THE IDENTIFICATION OF PATIENTS AT RISK

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Lipoprotein a (Lp(a)) can interfere with clot lysis and be deposited in the artery wall. When present in high levels, it represents a major risk factor for the development of atherosclerosis and coronary heart disease. The large differences in Lp(a) levels observed between individuals are largely due to hereditary factors and cannot be controlled with dietary or lifestyle changes. However, identifying at-risk individuals can be useful in alerting them to the need to eliminate or control other high-risk factors. Following the internalization of the Lp(a) assay, our service carried out a comparative study on serum samples.

Abbott Laboratories' Alinity C system, an automatic multi-parameter Chemistry and Immunochemistry analyzer, uses Turbidimetry/Immunoturbidimetry for the measurement of the parameter under study. The analytical performance of the Lp(a) reagent was evaluated by the correlation between Lp(a) from the external laboratory and Abbott's Lp(a) - 01R1420, in 32 samples in which it was requested from November 23 to December 26, 2023. Informed consent was waived because patient samples were used and personal information was excluded in this study.

The analysis of the performance evaluation data was performed in Microsoft Excel and SPSS version 27 for method comparison tests. In the presentation of the results, a descriptive statistical analysis was performed, highlighting the number of samples (N), the mean, the minimum and maximum values, and the standard deviation. The results of Lp(a) were correlated and there was no statistical difference between two methods using Deming's linear regression, for a 95% CI a statistically significant correlation was obtained with R2 of 0.9944 with a Pearson coefficient of 0.9972. The reagent showed good analytical performance and was practically superimposed on the results provided by the external laboratory.

It is expected that, with the realization of this parameter with the version of Clinical Chemistry reagents launched by Abbott® in the Alinity C system to be carried out in our service, it will improve analytical sensitivity, facilitate the daily routine of the laboratory and reducing the response time for the clinician.

P86

UNDERSTANDING POTENTIOMETRY: NA+ AND K+ EXAMPLE

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Introduction: In terms of Na+ and K+ values, the validation of the analytical results requires especial attention, since significant deviations from the reference values of these analytes may reflect serious clinical conditions that must be acted upon quickly.
Hyponatremia, for example, is a very common electrolyte disorder, which is life-threatening, when there is a rapid reduction of these levels, by causing brain edema, as it can be clinically insignificant. Variations of potassium levels are also frequent, like hyperkalemia, a severe clinical condition, since it can cause fatal cardiac arrhythmias. Considering the importance of these results, we found it relevant to confirm with a second reliable method available at our laboratory.

Objectives: Investigate the correlation between the results of two methods: the Alinity-c®, our routine method, and GEM3000®, regarding Na+ and K+ values.

Materials and methods: A total of 91 serum samples were analyzed using Alinity-c® and GEM3000®, to measure Na+ and K+ values. Both instruments use potentiometry.
GEM3000® uses direct potentiometry, where Alinity-c® uses indirect potentiometry. This last one uses the ICT reference solution to compare its potential measurements with the ones from the samples. The results were then assessed using MicrosoftExcel® for the statistical treatment. Linear regression and Pearson´s correlation coefficient were used to analyze the data.

Results: Regarding Na+ values, the following Pearson´s correlation coefficient and linear regression equation, respectively, were obtained: r = 0,931; y = 0,9196x + 12,123 (R2 = 0,865). For the K+ values, the comparison of the two methods showed a Pearson´s correlation coefficient of 0,989 and the linear regression equation obtained was y = 0,9559x + 0,3398 (R2 = 0,978).

Conclusions: The results showed that the two methods may be interchangeable, based on the significant correlation for both Na+ and K+ values (r = 0.931 and r = 0.978, respectively). Applying these results to our daily practice, it allows us to use the GEM3000® to confirm certain results from the Alinity-c®, that significantly escape from the normal range and may evoke some doubts concerning, for example, an error of pipetting, since it is a fast, simple method, which is available in our laboratory. This is especially important for analytes that have a great impact on clinical decision making and in which a fast response is crucial to prognosis, as is the case of Na+ and K+ values.

P87

AGEING BIOMARKERS: MAIN MOLECULAR, CELLULAR, AND PHYSIOLOGICAL INDICATORS

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Introduction: Ageing is a complex biological process characterized by molecular, cellular, and physiological changes contributing to losing homeostasis and increased vulnerability to diseases. Identifying reliable biomarkers is essential for assessing biological ageing and monitoring interventions.

Objective: To conduct a literature review on the main ageing biomarkers currently in use, highlighting their clinical relevance and evaluation methodologies.

Materials and Methods: A comprehensive search was conducted in scientific databases (PubMed, Scopus, and Web of Science) using the terms "ageing biomarkers", "biological ageing", and "senescence markers". Articles published in the last 10 years were included, focusing on biomarkers validated in clinical and experimental studies. Both ageing and ageing were used as the terminology varies from author to author.

Results: The most frequently identified biomarkers were classified into four main categories:

1. **Molecular:** Epigenetic clocks (Horvath Clock, DunedinPACE, GrimAge), telomere length, and microRNA expression (miR-34a, miR-146a).
2. **Cellular:** Cellular senescence markers (p16^INK4a, senescence-associated β-galactosidase), mitochondrial dysfunction (reduced efficiency of the respiratory chain, increased reactive oxygen species - ROS), and decreased autophagy (reduced LC3, Beclin-1)
3. **Physiological:** Reduced pulmonary capacity, decreased muscle (grit) strength, slower gait speed, and reduced heart rate variability.
4. **Biochemical:** Increased inflammatory cytokines (IL-6, TNF-α), oxidative stress markers (malondialdehyde, 8-hydroxy-2'-deoxyguanosine), PCR and hormonal changes (DHEA-S, IGF-1). Proteomic and Metabolomic Profiles and Signatures.

Conclusion: Biological ageing is best assessed through an integrated approach that combines molecular, cellular, physiological, and biochemical biomarkers. Epigenetic clocks are emerging as robust predictors of biological age, while mitochondrial dysfunction, oxidative stress, and reduced autophagy reflect fundamental ageing mechanisms. The combined use of these biomarkers represents a promising strategy for evaluating ageing progression and the effectiveness of interventions promoting longevity.

P88

EVALUATION OF NT-PROBNP AND D-DIMER LEVELS IN AQT90 FLEX ANALYZER AND RAMP 200 ANALYZER: A COMPARATIVE ANALYSIS

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Objective: To compare the obtained results of NT-proBNP and D-Dimer from the two analyzers used, Radiometer AQT90 analyzer and Response Biomedical RAMP 200 analyzer.

Material and methods: In this study we used Radiometer AQT90 analyzer – immunoassay technology and time-resolved fluorometric detection - and Response Biomedical RAMP 200 analyzer - immunoassay technology.
We used 48 patient EDTA whole blood samples to compare NT-proBNP results and 44 patient EDTA whole blood samples to compare D-Dimer results.
We used the linear regression on an Microsoft Excel Sheet.

Results: On the linear regression we observed a correlation of R2=0,977 for NT-proBNP and R2=0,658 for D-Dimer, and the percent differences average (AVG) of 50% for NT-proBNP and 47,6% for D-Dimer.
The European Biological Variation Database (EFLMBV), reveals that population biological variation (CVg) for D-Dimer is 35,4%. It also determined that intraindividual within-subject biological variation (CVi) for D-Dimer is 25,2%. The EFLMBV has no data for NT-proBNP.
The SEQC (Sociedad Española de Medicina de Laboratorio) reveals that population biological variation (CVg) for NT-proBNP is 16%. It also determined that intraindividual within-subject biological variation (CVi) for NT-proBNP is 10%.

Conclusion: The results of the analytical comparison show good analytical concordance between both methods for NT-proBNP determination. For D-dimer determination there is a very low analytical concordance between these methods.
The %AVG difference of results for NT-proBNP is above the respective CVi, which means that is not safe to use both methods for the monitorization of patients. The good analytical correlation suggests that RAMP NT-porBNP will have the same quality as AQT90 to monitor NT-proBNP on patients. However, the CVi used to define clinical safety when using both equipments for monitoring the same patient was last updated in 2020. So these results should be confirmed with more recent values as far as they come out.
The %AVG difference of results for D-Dimer is above the respective CVi, which means that is not safe to change the method used for monitoring the same patients.

P89

STABILITY STUDY OF FOLIC ACID MEASUREMENT IN PROTECTED AND UNPROTECTED TUBES EXPOSED TO VISIBLE LIGHT

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Folic acid (FA) is an essential vitamin compound for human health, playing a crucial role in the prevention of megaloblastic anemia and neural tube defects, as well as having significant implications for cardiovascular diseases and cancer.
FA measurement in the Abbott Alinity system is performed using chemiluminescence (CMIA) technology with the Abbott Alinity i Folate Reagent Kit (REF 08P14).
FA is sensitive to ultraviolet (UV) light, which affects its stability, and the protocol recommends protecting samples from light, requiring the collection of a dedicated tube for this purpose. To evaluate FA measurement in S-Monovette® biochemistry tubes, both PROTECTED and UNPROTECTED from visible light, and compare their stability and performance.

In this study, FA measurement was assessed in 35 randomly selected routine laboratory samples. The samples from both PROTECTED and UNPROTECTED tubes were analyzed in parallel using the commercial kit Alinity i Folate Reagent Kit (Abbot).
A total of 12 samples were selected for measurements at four different time points over 48 hours to assess the stability and variability of the assay.
Data recording was done using Microsoft Excel, where descriptive analysis was performed.
IBM SPSS Statistics version 27.0 was also used for parametric analysis, applying the paired t-test to determine whether there were significant differences in sample means, including over time measurements, with a statistical significance threshold of 5% and a 95% confidence interval (CI). Statistical analysis demonstrated a strong positive correlation ($r=0.996$; $p<0.001$) between PROTECTED and UNPROTECTED TUBES, with no significant differences between them (t-value=0.101, df=34, $p=0.920$). Regarding the impact of time on FA determination, the paired t-test results showed that UNPROTECTED TUBES did not exhibit significant changes over 48 hours ($p>0.05$). However, in PROTECTED TUBES, an initial decrease in FA values was observed within the first 24 hours ($p<0.05$), although no overall difference was detected between the groups.

This study confirmed that visible light exposure does not interfere with FA measurement using this methodology. Additionally, the findings suggest the potential for optimization and cost reduction in sample collection by eliminating the need for an additional dedicated tube for FA testing.

P90

ALLERGY TO HYMENOPTERA VENOMS: DIAGNOSIS OF NEW SUSPECTED CASES

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Insect venom allergy (HVA) is the leading cause of anaphylaxis in adults in Europe and can be fatal. It is estimated that between 60% and 95% of people have been stung by insects at least once in their lifetime.

A precise, quick, and accurate diagnosis to recognize IgE-mediated sensitization, allowing effective treatment with immunotherapy—which is considered curative for most patients—is essential.

The aim of this study was to analyze specific IgE for Hymenoptera venoms in 2024 in our ULS. The primary focus was to identify sensitization patterns and cases of multiple reactions.
Specific IgE tests were conducted for the venoms of Apis mellifera, Vespula spp., Polistes spp., and Vespa crabro using the ImmunoCAP® Specific IgE (kU/L) - Thermo Fisher® - Fluorescent Enzyme Immunoassay (FEIA) method.

A total of 172 people were tested, including 120 men and 52 women. Data analysis revealed that reactions to Hymenoptera venom predominantly affect men, particularly in younger age groups (0-17 and 18-34 years), possibly due to greater exposure to outdoor activities. The 50-64 age group was the most vulnerable to multiple reactions, with 23 affected individuals, highlighting the need for early diagnostic strategies.

Apis mellifera venom had the highest number of positive cases (123, 71.5%), with a male predominance of 70.7%. Vespula spp. venom showed 60 positive cases (34.9%), with 76.7% of affected individuals being men. For Polistes spp., 58 individuals tested positive (33.7%), with 81.0% of cases occurring in men. Finally, Vespa crabro had the fewest positive cases (42; 24.4%), with a male predominance of 78.6%.

An analysis was also conducted on individuals with sensitization to more than one venom. A total of 65 individuals presented multiple reactions, with the highest concentration of cases in the 50-64 age group (23, 57,5%), followed by those aged 65 or older (16, 69,6%). Male predominance was also observed in this group, with most cases occurring in older men.

IgE testing via FEIA is essential for detecting Hymenoptera venom sensitization, but complementary tests improve diagnostic accuracy.
Methods such as IgE inhibition assays, molecular component tests, and immunoblotting help distinguish genuine sensitization from cross-reactivity, allowing for more personalized treatment.

This study improves understanding of Hymenoptera venom reactions, emphasizing early diagnosis, prevention, personalized treatment, and complementary methods for better diagnostic accuracy.

P91

CONGENITAL SYPHILIS – THE IMPORTANCE OF ANTEPARTUM SCREENING

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Congenital syphilis is a preventable infection caused by the Treponema pallidum (TP) parasite from the Spirochaetaceae family transmitted from a pregnant individual to the foetus. Vertical transmission can occur transplacentally or during childbirth, from mothers with syphilis.
Women most likely to have children affected by congenital syphilis are those untreated for primary, secondary, or early latent syphilis.

Unfortunately, a lack of surveillance before or during pregnancy can lead to unnoticed infections until later in pregnancy or delivery. According to official European data, the incidence of congenital syphilis has been increasing.
This study aims to document the laboratory results obtained in newborns whose mothers tested positive for syphilis during pregnancy in 2024 at our center.

Methods: Syphilis screening was performed using the reverse screening algorithm.
All pregnant women undergo syphilis screening at their first antenatal visit (first trimester). In high-risk cases and regions with high prevalence, serology is repeated in the third trimester (28–32 weeks) and at delivery. If no prior test is documented, screening is performed at delivery.
Syphilis antibodies (IgG + IgM) are detected using chemiluminescence immunoassay (TP-CMIA) on the Allinity® (Abbott) system.
In seropositive mothers, newborns are tested with RPR/VDRL (quantitative), TPPA/TPHA (quantitative), and anti-treponemal IgMEIA (19S-IgM FTA-abs or IgM immunoblot).

Results: Among 610 pregnancies, 7 (1.15%) tested reactive for syphilis. One pregnancy was voluntarily terminated, and another was lost to follow-up. Of the five who continued care, four received appropriate treatment, while one did not.
At birth, all five mothers and newborns tested reactive. The infant of the untreated mother, classified as high risk, received a full 14-day penicillin course. Among the treated mothers, three newborns received a single prophylactic dose, while one began a 14-day treatment regimen due to an RPR titre four times higher than the mother's. The treatment was discontinued at day 10 by clinical indication.

Conclusion: All neonates were born at term with normal Apgar scores assessed at one, five, and ten minutes after birth. No immediate sequelae were reported at the time of discharge.
Universal antepartum laboratory screening is widely recommended, as screening followed by treatment with appropriate antibiotics typically prevents adverse maternal and neonatal outcomes.

P92

SEVERE HYPOGLYCEMIA – INTERFERENCE OR MEDICAL EMERGENCY?

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Introduction: Hypoglycemia in adults can be defined as a fasting glucose level below 70 mg/dL. In our centre, it is considered critical below 50 mg/dL in men and 40 mg/dL in women and has to be reported to the clinician as soon as possible. Extremely low levels (<10 mg/dL) are rare and often the result of preanalytical interferences or errors.
This work describes a clinical case that highlights the proactive role that Clinical Pathologists (CP) can play regarding the confirmation of in vivo critical results and prompt communication with clinicians, with high clinical impact.

Clinical Case: The case concerns a 73-year-old, autonomous man with a history of stage IV colon adenocarcinoma with liver and lymph node metastisation. He was admitted to our hospital for palliative surgical procedures to relieve cholestasis.

During this prolonged hospitalisation, he eventually developed cholangitis and later sepsis. At this point, his inflammatory parameters and cholestasis worsened. In addition, he had a glycemia level of 7 mg/dL.
Although the analytical phase has the fewest errors, the CP's first step was to confirm the result using a different equipment and methodology.
Glucose value was inferior (5 mg/dL) and serum pH was 7,44. In the meantime, the clinical file was consulted to check for signs and symptoms compatible with hypoglycemia, as well as possible causes. The patient was drowsy and sluggish, tachycardic and hypotensive. Previous glucose values were in normal ranges, and the patient had no prior history of diabetes mellitus. Additionally, leukocytes in blood count were 34700/µL.
The clinician was contacted and hypoglycemia was confirmed in capillary blood. The patient was administered glucose IV infusion and his condition improved.

Discussion: This patient was hypoglycemic, but other factors may explain the extreme low serum glycemia. Serum's pH suggested a delay in the preanalytical phase and blood count revealed leukocytosis. These findings suggest a significant consumption of glucose by glycolysis. This case highlights the active role that a laboratory service can play in patient management. Interpretation and confirmation of analytical results and immediate communication with clinicians can significantly shorten the initiation of therapeutics, with an important impact on prognosis.

P93

ERYTHROCYTE FOLATE VERSUS SERUM FOLATE: CLINICAL RELEVANCE IN LABORATORY EVALUATION

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Introduction: Folate, a water-soluble B vitamin, plays a key role in DNA synthesis, cell division, and hematopoiesis. Folate deficiency is associated with megaloblastic anemia, pregnancy complications, and cognitive decline. Laboratory assessment of folate status is commonly performed using serum folate, which reflects recent dietary intake, or erythrocyte folate, a long-term marker of folate stores. However, the routine measurement of erythrocyte folate remains controversial due to technical and analytical challenges.

Objective: To evaluate the clinical relevance of erythrocyte folate measurement compared to serum folate in diagnosing folate deficiency, with a focus on diagnostic accuracy, practical application, and laboratory feasibility.

Materials and Methods: A literature review analyzed peer-reviewed studies and clinical guidelines comparing the diagnostic performance, advantages, and limitations of both methods.

Results: Erythrocyte folate is a more stable and reliable biomarker than serum folate, as it is less affected by short-term dietary fluctuations and better reflects long-term folate status and metabolic reserves. However, its use in clinical practice remains limited due to pre-analytical handling difficulties, complex sample preparation, lack of standardization across laboratories, and higher analytical variability. These challenges contribute to discrepancies in reference ranges and interpretation between different methods. In contrast, serum folate remains the preferred test, given its ease of measurement, availability, and standardization in clinical laboratories, despite being strongly influenced by recent dietary intake and potential day-to-day variations. Some guidelines support erythrocyte folate as a superior long-term indicator, particularly in hematological disorders such as myelodysplastic syndromes, where fluctuations in serum folate may not accurately reflect intracellular folate levels. However, the broader clinical use of erythrocyte folate remains debated, primarily due to technical and logistical constraints in routine laboratory practice.

Conclusions: While erythrocyte folate measurement provides a more accurate indication of long-term folate reserves, its clinical utility is constrained by challenging analytical methods, and serum folate remains the primary diagnostic method. Further standardization of erythrocyte folate assays and clinical studies are needed to define its potential role in routine diagnostics.

P94

TO BE OR NOT TO BE A LABORATORY ERROR – THE STORY OF A DAY IS THE STORY OF A LIFE

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Introduction: The clinical laboratory plays a very important role in patient health care, as test results impact more than 60% of medical decisions. In the complete approach to quality and safety, monitoring the performance of the processes is assumed to be crucial. The overall response time – Turn Around Time – continues to be a fundamental indicator, universally used as a way of generically classifying the good laboratory's functioning. Throughout the process, errors may occur that lead to delay in the results. As we all know, the pre-analytical phase takes over most laboratory failures and a well-documented and recorded non-conformities aims of applying corrective actions, evidence based. In a fully automated laboratory, this context takes on a new meaning – the need for control and action.

Objective: Identify the main causes responsible for delay in sample results in a fully automated laboratory.

Method: Observational analysis of non-conformities responsible for the delay in sample results, in a working day. The failures were grouped into the categories: problems with samples, problems with equipment/analyzers, informatic problems, problems with human resources. The observation area was reception/input-output module in the automated track (a3600® Accelerator track - Abbott®).

Results: A total of 1765 samples were observed, of which 2.5% (44) showed errors. The two most prominent failures were sample problems (34%) and informatic problems (34%).

Equipment/analyzers accounted for 25% and the human failures, related to lack of reagents, 7%. Sample problems were mainly related to the barcode labels position and the incomplete coagulation process (appearance of fibrin). In the equipment/analyzers issues group, an aliquoter issue was the cause of delay in the observational day. Informatic issues were mainly related to no petition integration.

Conclusion: Analyzing the results, 75% of failures were avoidable. Informatic errors are too heavy and are directly associated with one of the main sources of delay in the laboratory, demanding intervention. Sample failures require training and information. Assuming an equipment malfunction on the day of observation, the remaining errors are kind of recurring ones so that we can simply extrapolate that one day (of observation) can correct a life in the laboratory. In conclusion, a periodic review of non-conformities must be carried out with the aim of evaluating the operational status and applying effective corrective actions.

P95

HIV TRANSMISSION IN THE CONTEXT OF THE CRIME OF SEXUAL ASSAULT: CASE REPORT

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Background: The Human Immunodeficiency Virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), remains a global public health challenge.

1. This infection weakens the immune system and persists throughout an individual's lifetime. HIV is primarily transmitted through sexual contact. Antiretroviral therapy (ART) suppresses viral replication, reducing the viral load to undetectable levels and preventing transmission
2. Despite a decline in new HIV and AIDS cases, Portugal remains among the Western European countries with the highest infection rates
3. HIV diagnosis relies primarily on detecting antibodies against viral proteins (anti-HIV-1 and anti-HIV-2); however, these may take weeks to develop post-infection. Most HIV-positive individuals are unaware of their status due to absent or nonspecific symptoms. Early diagnosis is possible through simultaneous detection of HIV-1 p24 antigen and antibodies
4. Additionally, nucleic acid testing (NAT) identifies viral RNA, confirming active replication
5. A negative NAT result indicates no detectable circulating virus at the time of analysis.

Objective: This study evaluates the possibility of HIV transmission by an HIV-positive individual accused of sexual assault, considering their virological status at the time of the event.

Methods: A venous blood sample was collected, followed by serum separation via centrifugation.

A 4th-generation enzyme immunoassay (EIA) was performed on the miniVIDAS immunoassay analyzer to detect HIV-1/2 total antibodies and HIV-1 p24 antigen. Additionally, real-time PCR was used to detect HIV RNA and assess viral load.

Results: Laboratory tests confirmed the presence of total HIV antibodies, indicating prior infection. However, neither HIV-1 p24 antigen nor viral RNA was detected, suggesting an undetectable viral load. This status is typically achieved after at least one year of ART and indicates the absence of active viral replication in the bloodstream.

Conclusion: The findings classify the individual as undetectable, meaning they were not infectious at the time of the alleged assault. Since treatment adherence is crucial for maintaining viral suppression, verifying ART compliance at the time of the event is recommended. Furthermore, periodic monitoring of viral load for up to 18 months is advised to ensure sustained viral suppression and confirm non-transmissibility.

Keywords: Antiretroviral therapy; Enzyme immunoassay; HIV; Sexual transmission; Viral load

P96

EVOLUTION OF PRENATAL SCREENING IN A CENTRAL HOSPITAL CENTER FROM 2015-2024

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Introduction: First and second trimester prenatal screening is offered to all pregnant women in order to select a high-risk population for the most common aneuploidies (trisomies 13, 18, and 21). The implementation of this screening has allowed for the use of invasive diagnostic methods, which were previously mainly used for pregnant women over the age of 35, to be directed toward a higher-risk group. On one hand, this has reduced unnecessary use in pregnancies with healthy fetuses from mothers over the age of 35, and on the other hand, it has improved detection in cases of fetuses with these trisomies from mothers under the age of 35. With the advent of non-invasive prenatal testing (NIPT), it was theorized that it could replace combined screening due to its higher specificity.Thus, this study aims to evaluate the evolution of the number of prenatal screening requests between 2015 and 2024, characterizing its population.

Materials and Methods: An anonymized search was conducted on the number of requests for first and second trimester biochemical screening and the age of pregnant women, from 2015 to 2024.

Results: During the studied period, an average of 2880 requests were made annually. An 1% increase was observed per year. On average, 2658 first trimester screenings and 221 second trimester screenings were performed.It was also found that the average and median age of pregnant women remained constant at 32 years, with a minimum age of 14 years and a maximum age of 51 years.

Conclusion: The number of prenatal screenings has been increasing, with this increase being consistent over the years, amounting to approximately 1%.

This increase is due to the growing number of first trimester biochemical screenings. In contrast, the number of second trimester biochemical screenings has remained relatively constant, making up only about 10% of the total annual screenings. Similarly, the studied population of pregnant women has maintained the average and median age of 32 years. Therefore, it is evident that the introduction of NIPT, despite its publicity, has not reduced the number of requests. Despite the specificity of this test, it has several limitations, and the most recent clinical guidelines support the combined use of prenatal screening followed by NIPT and/or invasive testing for the prenatal diagnosis of these aneuploidies.