



EVENTO ONLINE

**12ª REUNIÃO CIENTÍFICA
DA SOCIEDADE PORTUGUESA
DE MEDICINA LABORATORIAL**

29 A 31 DE OUTUBRO DE 2020

**PROGRAMA
&
ABSTRACTS**

12ª REUNIÃO CIENTÍFICA DA SOCIEDADE PORTUGUESA DE MEDICINA LABORATORIAL

29 A 31 DE OUTUBRO DE 2020

Mais informações em: www.spm.pt

PROGRAMA |

| 29.Out

16H00 – 17H00

SIMPÓSIO BECKMAN COULTER

CONTROLO TOTAL DO PROCESSO DE LABORATÓRIO COM OS NOVOS SISTEMAS DE AUTOMATIZAÇÃO

Moderador: **Mário Sá Carneiro** (Country Manager, Beckman Coulter)

Tom Coulson (European Product Marketing Manager – WORKFLOW & it SOLUTIONS, Beckman Coulter)

Santiago Prieto (Jefe del Servicio de Laboratorio Clínico. Hospital Universitario de Fuenlabrada, Madrid)

17H00 – 18H00

SIMPÓSIO SIEMENS

CONECTIVIDADE POINT-OF-CARE: UMA PONTE PARA A ACREDITAÇÃO E PARA O FUTURO

Moderador: **Andreia Dias** (Siemens Healthcare)

Felicity Dempsey

18H00 – 18H15 - PAUSA

18H15 – 19H15

SIMPÓSIO SPML

RASTREIOS NA GRAVIDEZ

Moderadores: **Patrícia Silva** (CHLO) | **Luísa Espinhaço** (CHEDV)

18h15 – 18h40 **Cláudia Teixeira** (CHUSJ)

18h40 – 19h05 **Carla Ramalho** (CHUSJ)

19h05 – 19h15 Discussão

19H15 – 20H15

SIMPÓSIO SPML

Moderadores: **João Pego** (CHUC) | **Amélia Almeida** (CHEDV)

19h15 – 19h40 **O PROLONGAMENTO DO APTT NA URGÊNCIA HOSPITALAR** | **Isabel Freire** (CHLO)

19h40 – 20h05 **TDM – DA MONITORIZAÇÃO À FARMACOGENÓMICA**

Maria Luís Cardoso (INSA) | **João Paulo Cruz** (CHLN)

20h05 – 20h15 Discussão

|30.OUT

09H30 – 13H00

CURSO SPML

Mediante inscrição prévia

ATUALIZAÇÃO EM PARASITOLOGIA CLÍNICA

Teresa Baptista Fernandes (CHLO)

09H30 – 13H00

CURSO SPML

Mediante inscrição prévia

APLICAÇÕES DA VARIABILIDADE BIOLÓGICA NO LABORATÓRIO CLÍNICO DO SÉCULO XXI

Jorge Pinheiro (CH Leiria)

16H00 – 17H00

SIMPÓSIO ABBOTT

Moderador: **Pedro Pereira** (Abbott)

16h00 – 16h25 **DIAGNOSIS OF SARS-CoV-2 DISEASE AND INFECTION**

Claudio Galli (Associate Medical Director Infectious Diseases | Abbott Diagnostics)

16h25 – 16h50 **CARDIOVASCULAR RISK STRATIFICATION IN APPARENTLY HEALTHY POPULATION AND IN COVID19 PATIENTS**

Stefano Favero (Medical Scientific Liaison Manager Europe | Abbott Diagnostics)

17H00 – 18H00

SIMPÓSIO THERMO FISHER

Moderador: **Nuno Franco** (Thermo Fisher)

TESTING FOR AUTOANTIBODIES IN THE DIAGNOSIS OF AUTOIMMUNE THYROID DISEASES

Christian Konrad

18H00 – 18H15 – **PAUSA**

18H15 – 19H15

SIMPÓSIO SPML

Moderadores: **Henrique Reguengo** (CHP), **Olga Carreira** (HGO)

18h15 – 18h40 **HEMOCROMATOSE** | **Graça Porto** (CHP)

18h40 – 19h05 **ESTADO DA ARTE** | **M^a José Teles** (CHUSJ)

19h05 – 19h15 **Discussão**

19H15 – 20H15

9^a CONFERÊNCIA PROFESSOR ALBERTO AGUIAR

Moderadores: **João Faro Viana** (CHLO), **Rui Farinha** (CHUSJ)

THE CENTRAL ROLE OF CLINICAL LABORATORIES IN COVID-19 PANDEMIC

Mario Plebani (UNIPD – Padova)

20H15 – 20H45

KEYNOTE LECTURE - ROCHE

O PODER DA ANÁLISE DE DADOS NA OTIMIZAÇÃO DA GESTÃO LABORATORIAL E HOSPITALAR

Roberto Gimenez Fernandez (Business Development Manager Roche)

|31.OUT

10H00 – 11H00

SESSÃO PRÉMIO POSTER SPML

Moderadores: **João Faro Viana** (CHLO), **João Tiago Guimarães** (CHUSJ)

11H00 – 12H00

SIMPÓSIO WERFEN

Moderador: **Margarida Guimarães** (CHULC)

ADAMTS13: O SEU PAPEL NO DIAGNÓSTICO DIFERENCIAL DA MICROANGIOPATÍAS TROMBÓTICAS

Teresa Fidalgo (CHUC)

12H00 – 12H15

LANÇAMENTO DO LIVRO

“QUALITY CONTROL OF QUALITATIVE TESTS FOR MEDICAL LABORATORIES”

Paulo Pereira (IPST)

12H15 – 12H30

ANÚNCIO DO(S) POSTER(S) PREMIADO(S)

COMISSÃO CIENTÍFICA

Adriana Pedrosa
Ana Paula Azevedo
Ana Paula Castro
Ana Paula Faria
Elsa Gonçalves
Eulália Costa
Fátima Vale
Fernando Rodrigues

Helena Brízido
João Faro Viana
João Mário Figueira
Manuela Ribeiro
Maria José Teles
Maria Luís Queirós
Rosário Luís

SOCIEDADE MEMBRO DE



EMPRESAS PATROCINADORAS



Cv's & RESUMOS



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Isabel Freire |

Cargos Actuais/Funções: Responsável técnica da bancada de coagulação do SPC do CHLO, EPE; Directora Técnica: Laboratório de Análises Clínicas do centro Clínico da GNR.

Experiência Profissional: Responsável técnica da bancada de coagulação do SPC/HSC (1987-1995); responsável pelos estudos especiais de hemóstase no SPC/HSC (1995-2008). Colaborou na Consulta de Anticoagulação Oral no SPC/HSC de 1987 a 1993 e no Instituto do Coração de 1988 a 2000. Fez parte da equipa do Lab Anal. Clin. Dr. Francisco Ferreira Crespo de 1988 a 31 de Dezembro de 2002. Fez parte da equipa de investigação UNICARV _ Unidade de Hemodinâmica e Intervenção Cardiovascular de 1986 a 2005.

Actividade Científica: 20 publicações; 42 Comunicações orais; 22 Posters; 11 reuniões- -Preletora; 25 Acções de Formação; Participou em 35 Ensaio clínicos.

PROLONGATION OF APTT TEST IN EMERGENCY LABORATORY

Unexpected prolongation of aPTT test in a hospital emergency room/lab may inadvertently be overvalued or neglected by pathologist and/or the clinician. What are the clinical/laboratory situations in which it should be explored? How important are complementary tests and/or the necessity to apply algorithms to help result validation? The importance of the communication between clinical and laboratory physicians. What are the clinical situations in which the study can and should be transferred to the routine hemostasis lab?

Maria Luís Cardoso |

Maria Luís Moral Westerman Cardoso is a senior pharmacist. She has a Master of Science in Human Genetics and a PhD in Biochemistry. Her career was mostly focused on the diagnosis, follow-up and molecular characterization of inborn errors of metabolism, which was her area of expertise. Presently she works as Pharmacist Geneticist at the Department of Health Promotion and Non-Communicable Disease Prevention of National Health Institute Ricardo Jorge (INSA) in Lisbon. As researcher her actual core of interests is precision medicine and pharmacogenomics, namely the genetic basis of adverse drug reactions.

orcid <https://orcid.org/0000-0002-8139-216X>

THE CONTRIBUTION OF LABORATORY MEDICINE FOR PERSONALIZED PHARMACOTHERAPY: PHARMACOGENOMICS AND THERAPEUTIC DRUG MONITORING

Maria Luís Cardoso¹, João Paulo Cruz²

¹*Departamento de Promoção da Saúde e Doenças não Transmissíveis, Instituto Nacional de Saúde Doutor Ricardo Jorge*

² *Serviços Farmacêuticos do Centro Hospitalar Universitário Lisboa Norte (Hospital de Santa Maria/Hospital Pulido Valente); IMed – Faculdade de Farmácia da Universidade de Lisboa.*

The aim of personalized pharmacotherapy is to ensure the right drug, to the right patient, at right dose, and right dosage interval: i) the selection of the right drug for a specific patient can be done pre-emptively testing for pharmacogenomics (PGx) biomarkers; and ii) therapeutic drug monitoring (TDM) can be used for selecting the right dose and dosage interval.

TDM involves measuring the concentration of drugs in biological matrices (moreover plasma). Then interpreting results (taking into account the most relevant clinical parameters) in order to guide posological adjustments; the objective is to maintain drug levels within a targeted therapeutic range or window for optimal patient benefit.

For many drugs including vancomycin, antiepileptic drugs, immunosuppressive drugs, cytostatics, TKIs, antiretrovirals, biologics for inflammatory bowel disease, there is a stronger relationship between plasma drug concentration and response than between dose response.

PGx is a complementary tool to TDM on personalised drug therapy, enabling drug selection and dose adjustment based on patient genetic background.

The majority of pharmacogenes studied in the context of PGx testing, code for metabolizing enzymes or membrane drug transporters influencing drug pharmacokinetics. The identification of variants in these genes enables patients categorisation as poor, intermediate, extensive and ultra-rapid metabolisers. In less extent, genes coding for drug targets, pharmacodynamics, are also scrutinized.

The most relevant clinical applications of PGx, as well as worldwide used guidelines were established by the Clinical Pharmacogenetics Implementation Consortium (<https://cpicpgx.org/guidelines/>) and by the Dutch Pharmacogenetics Working Group (<https://upgx.eu/guidelines/>).

Therefore, the use of PGx-based personalized dosing guidelines and TDM can in many cases reduce the incidence of adverse drug reactions and increase the likelihood of successful treatment outcomes.

Graça Porto |

CHUP-HSA-Centro Hospitalar Universitário do Porto, Hospital Santo António
ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto
I3S- Instituto de Investigação e Inovação em Saúde, Universidade do Porto

Graça Porto graduated in Medicine in 1979 at the University of Porto. In 1989 specialized in Hematology/Transfusion Medicine at Santo António Hospital, Porto, where she holds, since 2010, the position of Chief Consultant. In 1994 got the PhD degree in Immunology and in 2005 the degree of Aggregated Professor of Physiopathology, both at the Abel Salazar Institute for Biomedical Sciences (ICBAS) of the University of Porto (UP). In 2003 she joined, as clinical researcher, the Institute for Molecular and Cellular Biology (IBMC) and there became responsible for the molecular diagnosis of hemochromatosis at the Center for Predictive and Preventive Genetics (CGPP/IBMC). Since 2011 is director of a translational research group on basic and clinical research in iron biology (BCRIB) at i3S, the Institute for Investigation and Innovation in Health of UP. In 2018 was appointed as coordinator for the Disorders of Iron Metabolism in the EuroBloodNet, the European Reference Network for rare haematological diseases.

HEMOCHROMATOSIS

The term Hemochromatosis defines a group of genetic disorders of iron overload, mostly inherited in an autosomal recessive mode, which can potentially result in impaired organ structure and function, primarily in the liver (1). By far the most common and well defined form is due to homozygosity for the p.C282Y variant in *HFE*, the so called HFE-related hemochromatosis. The rarer forms of the disease are due to mutations in other iron related genes, generally designated as non-HFE hemochromatosis. For a long time, the term hemochromatosis has generated some confusion amongst clinicians depending on whether the disease definition was centered on phenotypic or genotypic criteria. The great scientific advances for the last decades and growing knowledge about cellular and systemic iron homeostasis and its molecular regulation came to clarify the pathophysiology of all forms of iron related disorders, in particular hemochromatosis that now includes in its definition the fact that it is caused by a deficiency of hepcidin, either decreased production or altered hepcidin–ferroportin binding (2). This lecture offers an overview of hemochromatosis in its various aspects, from its definition and physiopathology to the most practical approaches for diagnosis and treatment, focusing on the concept of early case detection and disease prevention.

(1) *Eur J Hum Genet.* 2016 Apr;24(4):479-95

(2) *Nat Rev Dis Primers.* 2018 Apr 5;4:18016

Maria Jose Teles |

1. Graduate Assistant in Clinical Pathology, in charge of the laboratory of hematology and cytology, at São João Hospital Center, responsible for the area of red cell pathology
2. Belongs to the pediatric-hematology group at São João Hospital Center
3. Routinely participate in hemochromatosis consultation in Porto Hospital Center
4. Member of the European Hematology Association – Scientific Working Group (EHA-SWG) of red cell and iron
5. Technical and scientific responsible for the area of the red blood cell in Center for Predictive and Preventive Genetics (CGPP), Institute for the Health Research and Innovation (i3S)
6. Member of the Scientific Committee of the Anemia Working Group Portugal (AWGP)
7. Member of the Scientific Committee of the Portuguese Patients Association of Hemochromatosis
8. Member of the group of Basic and Clinical Research on Iron Biology (BCRIB), Institute for the Health Research and Innovation (i3S)
9. Member of the EpiUnit, Institute of Public Health of the University of Porto (ISPUP)
10. Assistant, Clinical Pathology and Laboratory Medicine, Medical Faculty, University of Porto
11. Assistant, Clinical Hematology, Medical School of Minho University
12. Professional and Society memberships: Portuguese Society for Laboratory Medicine, Portuguese Society of Hematology, Portuguese Society of Pediatrics, Portuguese Society of Pediatric Hematology and Oncology
13. Lectures as qualified medical specialist in Pathology and Hematology
14. Novartis-Farma qualified medical specialist consultant



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POSTERS

P01 |

A CASE OF CARCINOMATOUS MENINGITIS

Rita Paulino¹, Karanini Ferreira¹; Maria Jorge Arroz¹; João Faro Viana¹

¹CHLO - Hospital de São Francisco Xavier – Lisboa

Introduction: Leptomeningeal metastasis (LM), also known as carcinomatous meningitis refers to the spread of malignant cells through the cerebrospinal fluid (CSF) space as a complication of advanced cancer. The most common solid tumors involved are breast and lung cancer, melanoma and gastrointestinal malignancies. Patients can present with a broad range of signs and symptoms. There must be a high suspicion for diagnosis, which is made using neuroimaging and CSF analysis.

Case summary: A 51-year-old male was admitted to the emergency room with complaints of headache, nausea, photophobia and dizziness. Physical examination revealed psychomotor lentification, aggressive speech and dysarthria.

The first CSF sample showed high protein concentration (114 mg/dL), low glucose (45 mg/dL) with no pleocytosis. Contrast-enhanced magnetic resonance imaging (MRI) revealed a diffuse leptomeningeal process probably related to a granulomatous (sarcoidosis) or tumoral process (lymphoma/carcinomatosis).

Three days later, another sample evidenced 53 cells/ μ L, with predominant mononuclear cells and some large non-hematological cells with poorly defined irregular nucleus, some hyper-basophilic and grouped in clusters.

This time flow cytometry (FC) was requested and identified 25.8% of cells compatible with carcinoma metastasis. Pathology identified the malignant cells as lung cancer metastasis by immunohistochemistry (TTF1), a day after the patient died.

Discussion: While positive cytology is highly specific, it is not as sensitive as neuroimaging abnormalities. If there is a high suspicion at least two different samples should be analysed. FC is extremely valuable in the diagnosis of infiltrating hematologic malignancies but is usually not informative for solid tumors. At our institution the identification of epithelial cells is being positive for the BER EP4 monoclonal antibody and negative for CD45 (pan leukocyte marker).

Take home message: Flow cytometry is more accurate to detect carcinoma cells in biological fluids, namely CSF, when compared to cytology. The availability of both approaches in a Clinical Pathology Department is clearly beneficial to direct the diagnosis in a few hours, as documented in this case.

P02 |

HOW FULL AUTOMATION MODEL AND SAMPLE TRACEABILITY WITH MINI-INDEXOR SYSTEM IMPROVED PATIENT CARE IN ONCOLOGY

Eulália Costa¹, Lucília Araújo¹; Fátima Amado¹; João Frade¹; Gilberto Marques¹; Fernando Rodrigues¹

¹Centro Hospitalar e Universitário de Coimbra

Introduction: The implementation of full automation models (FAM) in the clinical laboratory allows the consolidation of chemistry and immunochemistry analyzers in the same technological solution. These solutions allow biochemical (BC) parameters and tumor markers (TM) to be processed in the same sample in a single workflow without the need for aliquots or batch processing. The mini-indexor systems (MIS) manage the traceability of samples and the electronic integration of requests from the collection areas, avoiding registration on arrival at the laboratory and a variable pre-analytical waiting time

Aim: The aim of this study is to evaluate if MIS and the laboratory FAM improve patient care by delivering results in less time allowing the oncology treatments to begin earlier.

Material and methods: One-week global turnaround times (TAT) were measured for the oncology unit in 3 moments: 1) before FAM, with two sample flows for both BC and TM; 2) with FAM but without MIS in petition integration, with BC and TM in a consolidated way; 3) with FAM and MIS. All petitions between 8:00 and 10:00 were considered. The mean TAT included all the parameters: albumin, LDH, K, ALT, creatinine, CA15.3, Cyfra 21.1, CEA, CA19.9 and CA125.

Results: The TAT times were: 13h:54m in time 1 (n=123), 2h:58m in time 2 (n=282) and 1h:31m in time 3 (n=282). At this time petition integration occurs in the oncology unity and also includes sample transport.

Conclusions: The implementation of FAM in the laboratory improved TAT by 78,7%, since the results for BC and TM were consolidated in the same sample flow. After the introduction of MIS in the oncology unit the TAT reduction was 48,8%. These data strongly suggest that the MIS petition integration complemented the improvement of patient care provided with FAM, allowing patients to begin cancer treatments earlier (- 89,1%), return to the comfort of the home sooner and also contributes the hospital's resource optimization.

P03 |

CEREBROSPINAL FLUID WITH BONE MARROW ELEMENTS: CASE REPORT

Zuzana Melnik¹, Marta Costa Rego¹; Hugo Cruz¹; Inês Freitas¹; José Carlos Oliveira¹

¹Centro Hospitalar Universitário do Porto

Introduction: Bone marrow elements in cerebrospinal fluid (CSF) is rare. Increased cellularity and presence of myeloid/erythroid precursors in CSF may result in an erroneous cytological diagnosis of central nervous system infection or hemathological malignancy, when the specimen is actually not representative. We present a case of bone marrow contaminants in the CSF. The aim of this report is to create awareness of potential pitfalls and avoid erroneous diagnoses.

Case Report: A 52-YO woman with medical history of lumbar spine surgery, presence of interspinous device (L4-L5) and osteoligamentous fenestration (L3-L4) with recurrent lumbar back pain, was evaluated by neurology in outpatient clinic, for recent onset mild cognitive impairment. Patient underwent lumbar puncture (LP) for neurodegenerative disease suspicion. CSF sent for cytology examination was slightly blood-tinged. Cell counting was performed in a Neubauer chamber and one cytopspin slide was prepared and stained with Leishman-based stain for examination. The light microscopic observation of CSF revealed presence of myeloid and erythroid precursors at various stages of maturation as well as leucocytes and erythrocytes. One megakariocyte was observed. Patient's parameters of blood count were within normal limits. Reviewed by an experienced cytopathologist, facing normal hemathological results paired with patient's medical history, the diagnosis of bone marrow elements as contaminants in the CSF was suggested and the CSF was taken as non-diagnostic.

Conclusion: The common explanation for having bone marrow elements in CSF usually involves LP needle pushed too far anteriorly into the marrow cavity of a vertebral body, with concomitant sampling of bone marrow elements. This is usually due to decreased bone density linked to geriatric conditions, metastatic diseases or growing bone. A post-surgery anatomical change might as well have contributed to the sample contamination. A cytopathologist should be aware of this possible pre-analytical error when assessing cellular CSF containing erythroid and myeloid precursors.

P04 |

"MYSTERY CLIENT" FOR THE PERFORMANCE EVALUATION OF THE PRE-ANALYTICAL PHASE IN PARASITIC MORPHOLOGY AREA (PNAEQ 2019)

Lúcia de Jesus¹, Catarina Ventura²; Cláudia Júlio²; Ana Cardoso²; Silvia Viegas²; Teresa Baptista Fernandes³; Guilhermina Moutinho⁴; Quirina Santos Costa¹; Ana Faria²

¹Faculdade de Farmácia, Universidade de Lisboa, iMedULisboa

²Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa

³Centro Hospitalar Lisboa Ocidental, Serviço Patologia Clínica, Laboratório Microbiologia Clínica e Biologia Molecular

⁴Instituto Universitário Egas Moniz, Portugal

Introduction: The National Program of External Quality Assessment (PNAEQ) includes, since 2007, a Pre-Analytical Phase Evaluation program. In 2019, questions in parasitic morphology area were introduced in Mystery Client survey.

Objective: Evaluate participant's performance in Mystery Client survey, simulating real situations, regarding the adequacy and coherence of the information provided to patient and clinician, regardless of the collaborator, day and time.

Methodology: The survey included two trials: Mystery Client A simulating a patient with questions for *Enterobius vermicularis* research and Mystery Client B simulating a clinician requesting information on *Plasmodium* spp. research. For each trial, 2 anonymous phone calls were made to the participants, at different periods. The percentage of discrepant responses between calls and their suitability was assessed, being assigned a score between 0 and 3 for Mystery Client A.

Results: For Mystery Client A, 16/19 results were validated and the percentage of different responses between calls was: sample collection instructions (31%); sample reception time (38%); possibility of sending information by e-mail (44%) and information available on the laboratory's website (38%). The score distribution was 76%, 12%, 6%, 6%, for scores 0, 1, 2 and 3 respectively. For Mystery Client B, 17/19 results were validated and the percentage of different responses between calls was: performing *Plasmodium* spp. research (6%); thin film preparation (12%); thick film preparation (18%); species identification (47%); parasite quantification (41%), and analysis performed in the laboratory or subcontracted (18%). Of all participants who declared to perform the analysis (16/17), 75% stated in both calls using thin and thick films procedure, 50% identify the species, 25% quantify the parasite and 13% perform all the procedures described.

Conclusions: The results of Mystery Client survey revealed a discrepancy between the information provided by collaborators on the 2 phone calls, the need to update knowledge regarding the collecting procedure for *Enterobius vermicularis* research, and the reference methodology for malaria diagnosis. The evaluation of the pre-analytical phase should continue in the field of parasitic morphology, in order to harmonize the information provided to patients, contributing to reliable laboratory results.

P05 |

INFLUENZA - STRATEGIES FOR RATIONALIZATION AND CLINICAL ADEQUACY OF LABORATORY PRESCRIPTION

Micaela Batista¹, Luís Roseta²; Lucília Araújo²; João Pereira-Vaz²; Vanda Mota²; Lurdes Correia²; Fernando Rodrigues²

¹*Instituto Português de Oncologia de Coimbra Francisco Gentil, EPE*

²*Centro Hospitalar e Universitário de Coimbra, EPE*

Introduction: Influenza A and B viruses are RNA viruses within the Orthomyxoviridae family, that present a seasonal behavior, occurring predominantly in the winter. They can cause asymptomatic infection or acute respiratory disease (flu), being therefore one of the biggest causes of morbidity and mortality, especially in risk groups (children, pregnant women, elderly, chronic and immunosuppressed patients).

Objective: To evaluate the impact of the implementation of measures to rationalize influenza virus research requests, in a tertiary hospital, in the 2019-2020 season.

Methods: All samples were collected by oro/nasopharyngeal swab and processed using Xpert® Flu/RSV Assay, using GeneXpert® equipment (Cepheid). The following measures were adopted to rationalize exam requests: restriction of the online request for research of Influenza virus (A and B) outside the period of the seasonal flu epidemic; availability of a survey based on clinical and epidemiological criteria to justify the request made, considering the DGS Standard 006/2019 and the weekly survey of the number of positive flu cases in order to determine the best time to stop the online order.

Results: 1804 flu tests were performed, after answering the online survey, in patients with clinical criteria or belonging to risk groups. There were 558 positive tests for respiratory infection of viral etiology (Influenza A, B and Respiratory Syncytial Virus), of which 333 were positive for Influenza A and 90 for Influenza B. In the 2019-2020 season, the peak of Influenza B occurred in the third week of December 2019 and the peak of Influenza A occurred in the first week of February 2020. It is expected that the outbreak will end in late February, given the downward trend shown.

Conclusion: The strategies adopted had an impact on organizational and resource management, given the decrease in the number of orders, with considerable impact on cost reduction by 30.7% compared to the same period last year, resulting in savings of 32,040 euros. The implementation of the survey allowed the clinical adequacy of the prescription of the flu test, acting as an incentive to the reflective clinic. Weekly surveillance and the reporting of positive cases to the national flu surveillance network also allowed for better control and assessment of the outbreak trend.

MODEL FOR THE COMPUTATION OF CUTOFF FOR “IN-HOUSE” OR MODIFIED TESTS

Paulo Pereira, Ph.D.¹, Sandra Xavier, Ph.D.²

¹*Instituto Português do Sangue e da Transplantação;*

²*Instituto Politécnico de Beja*

Introduction: The clinical decision point in qualitative tests is defined as the test threshold that differentiates positive from negative results, referred to as the “cutoff.” Its selection is required for “in-house” or modified tests. A modification should only occur in special and justified situations. A sensitivity-specificity tradeoff derived from the cutoff choice usually happens, i.e., an increase in sensitivity is accompanied by a reduction in specificity and vice versa. A “best” cutoff is associated with a “best” tradeoff for a claimed performance - “better” condition sensitivity or “better” condition specificity.

Objective: The presentation briefly introduces and discusses an approach to identify “the best” cutoff point based on the “the best” sensitivity-specificity tradeoff.

Material and methods: The receiver operating characteristic (ROC) curve design is a fundamental methodology for the identification of a cutoff that fits the purpose of the test. The overall efficiency is related to the area under the ROC curve (AUC), but it should be clear that this area can not be misunderstood with condition sensitivity or specificity, and it has very limited interest to define “the best” cutoff. We will assume a virology immunoassay for the screening of human T-lymphotropic virus types I and II (HTLV I/II) in a hospital medical laboratory. Numerical results are expressed in absorbance (A). 47 infected samples and 179 non-infected samples are used. A sensitivity of 100% and specificity $\geq 90\%$ are claimed (better specificity).

Results: 1000 hypothetical discriminators (percentiles) are considered. The criterion is met for 78 possible cutoffs from 0.713 A to 0.791 A (acceptable sensitivity-specificity tradeoff).

Discussion: The computation was done using spreadsheet software. The results indicate that the test meets the claimed requirements, suggesting a large number of possible cutoff points. So, what is “the best” point? What fits the objective of the test is 0.713 A, since, theoretically, it is least likely to be affected by a lack of sensitivity as it is the lowest value. Note that if the focus is a better specificity, “the best” discriminator should be 0.791.

Conclusions: ROC curve design is critical to a reliable estimate of “the best” cutoff point since selection is based on “the best” sensitivity-specificity tradeoff. Although the ROC curve concept is not systematically used in the medical laboratory, it should be encouraged.

CLINICAL CASE REPORT – HEMOGLOBIN AIX-LES-BAINS

Bruna Malheiro¹, Patrícia da Cunha Rodrigues¹; Marina Majar¹; André Ferreira Pinto¹; Alexandra Estrada¹

¹*Hospital de Braga*

Introduction: Glycated hemoglobin (HbA1c) reflects average plasma glucose over the previous 8 to 12 weeks, the lifespan of the erythrocyte. Measurement of HbA1c is widely used for the diagnosis and management of patients with diabetes mellitus. Boronate affinity, ion-exchange high-performance liquid chromatography (HPLC) and immunoassays are the most common methods for measuring HbA1c. The usage of HPLC to measure HbA1c has led to the incidental finding of hemoglobin (Hb) variants, some of which interfere with the measurement of HbA1c levels through this method. We report a case of a patient in which an Hb variant was found while measuring HbA1c.

Case description: A 41-year-old male patient was admitted to the emergency room in our Hospital due to acute myocardial infarction. In the follow-up Cardiology appointment, an analytical profile was requested, which revealed a fasting glycemia of 109mg/dl and an oral glucose tolerance test of 109mg/dl (0'), 159mg/dl (60') and 138mg/dl (120'). The measurement of the HbA1c utilizing the HPLC method with the HA-8180T analyzer revealed an approximate value of 23.1%, which was considered an error by the equipment software and the presence of a Hb variant was signaled. Due to the impossibility of identifying the Hb variant present through the methods available in our laboratory, a peripheral blood sample was sent to a reference laboratory, which detected the presence of the mutation c.17C>T; p.Pro6Leu in the heterozygous state. This mutation was detected through Polymerase Chain Reaction/Sanger Sequencing of the HBB gene, corresponding to the variant Hb Aix-les-Bains. The genes HBA1 and HBA2 were also sequenced and revealed no pathogenic variants.

Discussion: In this case, the patient is a carrier of the variant Hb Aix-les-Bains, which has no clinical significance. However, it interferes with the measurement of HbA1c through the HPLC method, so laboratories should be aware of the limitations of their method, as therapeutic decisions are often based on these measurements. Therefore, an alternative method for measurement of HbA1c should be utilized, such as boronate affinity. In diabetic patients who are Hb Aix-les-Bains carriers, glycemic control can also be monitored through serial blood glucose determinations, by determining fructosamine levels, or glycated albumin.

P08 |

DIAGNOSING MALARIA WITH NEW IMAGE IDENTIFICATION ALGORITHM PARASIGHT™ P-002

Luis Carvalho Rodrigues¹, João Godinho²; José Souza³

¹Hospital de S. Francisco Xavier (Centro Hospitalar de Lisboa Ocidental);

²Luanda Medical Center;

³Yapama

The WHO estimates that 500 million tests for diagnosing malaria are carried out annually and predicts that the number will continue to increase. Diagnostic methods generally available include microscopy, immunochromatography (RDT) and PCR. Microscopy detection limit is usually referred to as 250 parasites / ul for thin smears but this requires highly trained personnel and the limit worsens rapidly (until 600-1000 parasites / ul) with poorly trained personnel and fatigue. RDT has little sensitivity in cases with low parasitemia and performance is inconsistent between different brands and batches. PCR is expensive and technically demanding.

In recent years, a new device of image identification system emerged (Parasight™ P-002, Sight Diagnostics). Approximately 1.5 million red cells are stained and arranged in monolayer and then analyzed by a machine learning algorithm, which allows the detection, counting and species identification. The equipment is easy to maintain and operate. Several studies point to a sensitivity and specificity of 99% and 100% respectively compared to PCR, both for *P. falciparum* and *P. vivax*. Average Pearson correlation coefficients are of 0.84 for species identification when compared to trained microscopists.

We used one of these devices for two years in a clinical laboratory that serves the population of Luanda (Angola), where official statistics point to more than 1 million people infected each year. A total of 47 592 samples were evaluated, of which 87.4% were negative, 2.9% were positive and 8.4% were rejected. Of the positive samples, 85.4% were identified as *P. falciparum* (parasitemia between 100 and 99 340 /ul) and 14.6% as *P. vivax* / *ovale* (parasitemia between 100 and 9 740 /ul). Only in 3 cases (0.2%) did the equipment give a doubtful identification. Suspected positive were 1.3% of samples: in 57.7% it was pointed out that could be an high reticulocyte count. Of the rejected samples, 58.9% were attributable to operator errors and 41.1% to external vibrations or other unidentified causes that disturbed the optical reading.

For six months, all 101 positives with parasitemia <500 /ul (approximately 3 /field 400x) were reviewed by microscopy. Only 7 (6.9%) did not confirm the result and were negative under microscopy. In 2 cases there were Howell-Jolly bodies and in 4 cases there were platelets > 600 x10³ /ul.

P09 |

A CASE OF ACQUIRED HEMOPHILIA A IN AN ELDERLY MAN

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Introduction: Acquired Hemophilia A (AHA) is a rare bleeding disorder caused by autoantibodies directed against Factor VIII (FVIII). Although there are several etiologies, up to 50% of cases are idiopathic.

Aim: Study of a patient diagnosed with AHA.

Case Presentation: A 77-year-old male patient was admitted to the Emergency Department due to spontaneous and exuberant bruises and hematomas in the upper and lower limbs, with 3 weeks of evolution. No relevant personal or family history. No history of anticoagulant or antiplatelet drugs. Analytically he presented with hemoglobin 7.4g/dL, platelets 280x10⁹/L, Activated Partial Thromboplastin Time (APTT) 105 seconds and normal Prothrombin Time and Fibrinogen levels (Clauss). FVIII levels were 0.6%. The measurement of APTT in an immediate mixture of the patient's plasma with normal plasma after 2 hours of incubation at 37°C showed no correction. The titration of clotting factor inhibitors was positive for FVIII inhibitors – 34 Units Bethesda. The lupus anticoagulant test was negative and Von Willebrand factor levels were normal.

After diagnosis of AHA the patient was admitted and started immunosuppressive therapy (prednisolone and cyclophosphamide). During the first days of hospitalization spontaneous hematomas reoccurred and on the 8th day he started a new therapy with FVIII bypassing agent, thereafter the symptoms did not reappear. He presented a favorable evolution with discharge on the 36th day of hospitalization.

Discussion: AHA is more frequent in adults over 65 years-old. The clinical manifestations are variable often with severe hemorrhagic complications which rarely correlate with the titer of FVIII inhibitor or its residual activity. The therapeutic approach consists of bleeding control agents and immunosuppressants.

Conclusion: AHA is a disease with a heterogeneous clinical presentation and a challenging diagnosis. Advanced age at presentation, hemorrhage severity and the associated pathologies contribute to the high morbidity and mortality. Until the eradication of FVIII inhibitor, bleeding risk persists, and in order to reduce the serious or fatal bleeding risk treatment should be immediately instituted, regardless of inhibitor titer, residual FVIII activity or associated pathologies.

POSTERS EM EXPOSIÇÃO NA GALERIA

P10 |

LACTOSE TOLERANCE TEST REDUCED TO 60 MINUTES

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Introduction: Lactase is an enzyme that hydrolyzes lactose in glucose and galactose monosaccharides. It is expressed in the small intestine during the neonatal period. After childhood there are two possible phenotypes: one with lactase persistence and the other one with a decrease in enzyme's activity. If lactase is absent or in a low level, the non absorbed lactose molecules osmotically attracts fluids to the intestinal lumen. In addition, the lactose that reaches the colon is fermented by the bacteria, which can lead to various gastrointestinal symptoms.

There are several tests to assess lactase deficiency: genetic tests for detecting 13910C/T polymorphism, duodenal lactase activity by intestinal biopsy, expired hydrogen test and the lactose tolerance test (LTT), which assesses glucose uptake after lactose administration.

Objectives: The objective of this work is to evaluate if decreasing LTT to 60 minutes there is a change in its final result.

Material and methods: A retrospective study of LTT performed in a hospital laboratory between 2015 and 2019 was carried out.

To perform the LTT, a solution with 50g of glucose is administered and blood samples are obtained, for glucose determination, at fasting, 15, 30, 60, 90 and 120 minutes after administration (to exclude situations where the lactose test is not interpretable). In the same week, a solution with 50g of lactose is administered and blood samples are taken in the same periods (T0, T15, T60, T90 and T120).

Positive results (without lactase persistence) were considered when the increase in glucose was less than 20 mg/dl in relation to the fasting value in all of the measurements.

Results: During the study period, 45 LLT were performed. 1 test was excluded, because it was not possible to interpret the result.

Of the 44 results analyzed, 27 were positive and 17 were negative. In 100% of cases analyzed, eliminating the T90 and T120 measurements, the interpretation of the test did not change.

Conclusions: LLT of 1 hour, with fasting glucose measurements, at 15, 30 and 60 minutes seems to be a reasonable alternative, as it would decrease the patient's discomfort with a smaller number of phlebotomies and less time spent in the hospital. It reduces costs and allows more time for laboratory technicians and nurses to do other tasks.

P11 |

SUSCEPTIBILITY TO RUBELLA IN CHILDBEARING AGE WOMEN IN NORTH PORTUGAL

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Introduction: Rubella is a viral infection with serious consequences during pregnancy due to teratogenic effects. In Portugal, the last annual report revealed the rubella eradication in 2017. However, there are not more national recent data.

Aim: Determine the rubella immune status of childbearing age women and verify if there is a correlation between age and IgG average concentrations.

Material and Methods: It was performed a retrospective study, reporting data collected at the community laboratory from January 2017 to December 2019. This study included 5758 women, age 18-49, with the first prescription for evaluation of rubella immune status, using as exclusion criteria cases without IgG and IgM co-determination. The results were obtained by chemiluminescent microparticle immunoassay. The results were presented using frequency distribution for population age, Parts of the whole graph to IgG and IgM distributions and statistical data was performed by GraphPad 8.3.1, Mann-Whitney test for IgG concentrations and Simple linear regression to correlate age and IgG average concentrations.

Results: 86,23% of the cases present immunity to rubella (average= 32 years), and 13,77% susceptibility to rubella (average= 29 years). Although all constraints, there was an increasing tendency to the rise of the IgG concentrations with age. The group between 29 and 36 years registered the higher frequency of samples. In the limits groups, the IgG concentrations were the lowest up to 29 years and were the highest from above 36 years.

Discussion/ Conclusion: The higher percentage of rubella immune status is in agreement with recent World Health Organization data. Between 18-30 years it was verified lower values of IgG concentration that may be justified by the immunization conferred by two doses of rubella vaccine. Although the IgG concentration can be affected by individuals factors, the immunization by rubella infections confers higher IgG concentration than vaccination. Population-based seroprevalence studies should be carried out in the future to determine new cut off values, and all women in childbearing age should reinforce the vaccine if the susceptible status were determined in the preconception period.

P12 |

DETERMINATION OF PREANALYTICAL UNCERTAINTY FOR SEVEN CLINICAL CHEMISTRY ANALYTES IN SERUM

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Introduction: In the Medical Laboratory, uncertainty on the result may arise from various sources, including preanalytical, analytical and postanalytical phases. Accounting the highest percentage of error in the total analytical process in the medical laboratory, preanalytical phase is often overlooked as a source of variation/uncertainty. The identification and assessment of preanalytical variables is fundamental to determine its influences (uncertainty) in the final result, identifying improvement opportunities.

Objective: The aim of the study was to determine the combined (uc) and expanded (U) uncertainty associated to preanalytical phase variability, namely venous puncture, processing delay, refrigeration, freezing, transport and lack of homogenization, on seven clinical chemistry analytes, in serum samples: Albumin (Alb), C-Reactive Protein (CRP), Calcium (Ca), Cholesterol (Chol), Creatinine (Crea), Glucose (Glu) and Potassium (K).

Material/Methods: Blood was collected from each arm of 39 volunteers into 5 serum-separation tubes. The data pairs for each procedure were evaluated according to laboratory standard conditions and the experimental alternative and converted into total coefficient of variation (CV) values. Standard preanalytical uncertainty for each variable were obtained by subtracting the analytical CV from the total CV. Combined uncertainty (uc) was determined by incorporation of standard uncertainty for each variable. The samples were analysed on Siemens Advia®1800 Chemistry System.

Results: Expanded uncertainty for CRP, Glu and K were 20,40%, 11,26% and 6,42%, respectively. Regarding venous puncture, the most affected parameters were CRP, K and Glu. Refrigeration influences Crea and Ca values, whereas freezing affects all analytes. Alb and Chol levels were only affected by freezing conditions. Processing delay impacts PCR, Glu, K and Ca levels. Transport conditions alters CRP, K, Glu, Crea and Ca levels. The absence of tube homogenization affects CRP and K serum levels.

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

P13 |

TOTAL AUTOMATION MODEL PROMOTES STAFF SKILLS BY ELIMINATING TIME CONSUMING TASKS: THE CHUC-CORELAB EXAMPLE

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Introduction: In the clinical laboratory, full automation models (FAM) are implemented to manage large laboratory areas with a high volume of samples. The complete or partial automation of manual tasks allows the laboratory to obtain safety, reduce turnaround time, reduce collected tubes, simplify tasks and free professionals for other activities. In our laboratory the implementation of a total automation model that fully automates the pre-analytical, analytical and post-analytical steps released the lab technicians allowing availability for new activities.

Aim: The aim of this work is to evaluate the overall daily time that was previously consumed in repetitive tasks with no added value, in order to measure the impact of automation in the promotion of the professional's skills.

Materials and Methods: Workflows prior to FAM were identified and all processing steps were described. In the pre-analytical (after sample arrival) and pos-analytical phase 7 tasks, that were automatized with the FAM, were identified and the time dispended for each sample measured (in seconds-s): load centrifuge - 3s; unload centrifuge - 3s, decap - 1s; load in rack - 0,5s; load in archived rack - 0,5s; dispose - 0,5s. The total of samples in one month (October 2017) were used to calculate the total time dispended in these manual tasks.

Results: A total of 21482 samples were processed in the evaluated period. A total of 1h50m per day were calculated as daily time saved in activities that were automatized in the new FAM of our laboratory. This staff release allowed the professionals to adopt new tasks.

Conclusions: The time saved in the automatized tasks corresponds to 26% of the 7 hours lab shift, showing the strong impact of FAM in the laboratory human resources. This staff release allowed the professionals to adopt new tasks as process control, stocks management, professional qualification and review of the written procedures, for example, promoting the usage of all their potential and skills.

P14 |

CHANGES OF HEP-2 INDIRECT IMMUNOFLUORESCENT TEST OVER TIME: A CENTER EXPERIENCE

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Background: Antinuclear antibodies (ANA), as detected by the indirect immunofluorescence assay on HEp-2 cells (IIFA HEp-2), are important diagnostic markers in autoimmune diseases. However, ANA testing lacks specificity and positive results can occur in severe burns or viral infection and have been reported in healthy people, especially in older population.

Objective: To determine the evolution/seasonality of ANA testing over time.

Methods: Records of the past 5 years (2015, 2016, 2017, 2018 and 2019) were collected through ClinidataXXI® software. The data included reports on ANA testing (titers and patterns) sorted by month and patient's demographic characteristics.

Results: The laboratory tested 41466 ANA (IIFA HEp-2) over five years. The number of tests increased 34.9% between 2015 (n=7030) and 2019 (n=9481). Twenty two percent (n=9062) of the tests were follow up repetitions. January was the month with more requests. The departments with more requests were Rheumatology (25,1%), Internal Medicine (15,8%) and Gastroenterology (7,3%). The 41466 tests corresponded to 32404 patients, with 66,7% (21611) females.

The result was positive in 17,7% (7343) tests. The highest titers (>1/320) were observed in two seasonal heights: August and January.

The patterns observed: Nuclear in 6136 tests (84%), Cytoplasmic in 886 (12%), Mitotic in 32 (0,4%) and Nuclear plus Cytoplasmic in 289 (3,9%). Within Nuclear patterns: Fine Speckled in 32,4% of the tests; Homogenous in 28,2%; Coarse Speckled in 10,9%; Centromere in 5,1%; and Nucleolar in 4,9%. Within Cytoplasmic patterns, Reticular/AMA in 1,4% tests.

Regarding follow-up, 5591 patients repeated the test at least once. The number of patients with negative result in the first test was 74,4% (4159). Of those, 314 turned positive in subsequent testing. Positivity in the first test happened in 25,6% (1432) patients and 239 of these changed later to negative. Overall, 9,6% (537) patients showed an increase on their titer between first and last evaluation.

Conclusions: Demand for ANA testing keeps growing. Clinicians should be aware of the clinical suspicion for the respective patient when requesting ANA, because, despite its excellent screening ability, the test lacks specificity. In our analysis, ANA titers showed very little variation in subsequent follow-ups. This behaviour seems to be in accordance with other studies. The higher titers, observed in August, might be related with UV-light exposure, a powerful inducer of ANA.

P15 |

COLORECTAL CANCER SCREENING IN THE PORTUGUESE POPULATION: THE KEY TO SUCCESS

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Introduction: Colorectal cancer (CRC) is one of the leading causes of mortality worldwide. Screening programs using immunochemical methodologies to quantify hemoglobin in stool samples have been implemented in extended populations. It has some advantages associated as the early detection with a non-invasive test, low cost and simplicity when compared to imaging tests. A screening program has been implemented in the last 3 years, involving a population aged over 49, in the center of Portugal with the samples being fully processed in our laboratory. However, the success of a program of this dimension requires perfect alignment in all axes of intervention, including information to the patient, sample fulfillment, request, transportation, request integration, testing, results validation and informatic information availability in the primary health care centers (PHCC).

Aim: The aim of this study is to evaluate main causes of distress in a screening program in order to improve the successes the populational CRC evaluation.

Materials and Methods: A survey of the main associated problems was made since the beginning of the program (2017), namely the causes of sample rejection. Other problems related to logistics were also inventoried.

Results: A total of 45294 samples were received from the PHCC between december 2017 and january 2020. Of those, 3366 were rejected (7,0%). The main causes of rejection were: sample stability outdated (30,7%, n=1035), unclean collection device (40,0%, n=1346), excessively filled collection device (11,0%, n=371), poorly filled collection device (17,8%, n=578) and opened collection device without reagent (0,5%, n=16). Other non-quantified functional causes involve informatics issues between the central hospital and the PHCC.

Conclusions: A main cause for sample rejection is directly related to the sample collection (69,3%) while the quantified organizational issues account for 30,7%. Lack of accurate instruction to patient seems to be the greatest weakness in the implemented colorectal cancer screening and a decisive factor in the success of any population screening program.

P16 |

MONITORING AND PROCESSING HAEMOLYTIC, ICTERIC AND LIPEMIC SAMPLES IN EUROPEAN AND PORTUGAL LABORATORIES...CARE IS ALL WE NEED...

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Introduction: Laboratory results play an essential role in both medical decision-making and in patient management. For this reason, laboratory test results need to be of the highest possible quality. No guideline currently exists on how to detect or document haemolysis, icterus or lipemia (HIL) in blood samples, nor on subsequent use of this information.

Aim: An overview of the guidelines and recommendations provided by the European Federation of Clinical Chemistry and Laboratory Medicine Working Group for the Pre-analytical Phase (EFLM WG-PRE), through a survey across European medical laboratories including Portugal, for assessing current practices in HIL monitoring.

Materials and Methods: Based on a review of part two of two coherent articles of the EFLM WG-PRE, which covered practices on monitoring HIL. An online survey containing 39 questions dealing with a broad spectrum of pre-analytical issues, was disseminated to EFLM member countries. Seventeen questions exclusively focused on assessment, management and follow-up actions of HIL in routine blood samples.

Results: Valid responses from 37 countries were received. From a total of 1.265 laboratories, 1.160 (86%) of all responders stating to analyse blood samples monitored HIL. Of the 57 participating laboratories from Portugal, 49 (86%) monitored HIL. HIL was mostly checked in clinical chemistry samples. HIL detection by automatic HIL indices or visual inspection, along with haemolysis cut-offs definition, varied widely among responders. A quarter of responders performing automated HIL checks used internal quality controls. In haemolytic/icteric/lipemic samples, most responders (70%) only rejected HIL-sensitive parameters, whilst about 20% released all test results with general comments. Other responders did not analysed but rejected the entire sample, while some released all tests, without comments. Overall, 26% responders who monitored HIL were using this information for monitoring phlebotomy or sample transport quality.

Discussion: Strategies for monitoring and treating haemolytic, icteric or lipemic samples are quite heterogeneous in Europe. Portugal had a quite acceptable percentage of HIL monitoring. The WG-PRE will use these insights for developing and providing recommendations aimed at harmonizing strategies across Europe.

P17 |

HMGCR ANTIBODIES - A RARE ENTITY

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Introduction: Inflammatory myopathies are a group of systemic autoimmune diseases. Myopathy associated with anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibodies is a rare immuno-mediated necrotizing myopathy, characterized by myalgia and progressive weakness of proximal muscles. Association with statins and development of this entity is reported. Casuistry in our tertiary hospital is 2 cases in 2 years.

Case 1: Male, 72 years, admitted to the emergency department with myalgia and decrease of muscle strength of lower limbs with 4 weeks of evolution. One month before the onset of symptoms, pitavastatin with atorvastatin were started, as usual medication. Analytically, there was an increase in creatine kinase (CK): 8457U/L (reference value(rf)<171), glutamic oxaloacetic transaminase (TGO): 416U/L (rf<35); glutamic pyruvate transaminase (TGP): 496U/L (rf<45) and lactate dehydrogenase (LDH): 967U/L (rf<248). Electromyography (EMG) revealed muscle fiber injury. Study of specific myositis antibodies revealed anti-HMGCR antibodies. The patient underwent muscle biopsy that showed extensive necrotic areas and started corticosteroid therapy (CT). During hospitalization, symptoms and analytical parameters were stable, with a slight improvement after CT. Nonetheless, there was further worsening, with development of respiratory infection.

Case 2: Male, 59 years, admitted for etiological study after episode of rhabdomyolysis with elevated levels of CK: 8635U/L, TGO: 217U/L and TGP:196U/L. Within a month after the start of Atorvastatin, the patient began to lose muscle strength. The hypothesis of statin-induced myopathy was suspected and the medication was suspended, however the patient maintained symptoms and analytical changes. EMG revealed muscle fiber injury. Study of specific myositis antibodies revealed anti-HMGCR antibodies. Muscle biopsy showed muscle cell necrosis. CT was started, with clinical and analytical improvement.

Conclusion: Despite rare, hypothesis of myopathy associated with anti-HMGCR antibodies must be considered as differential diagnosis with other neuromuscular disorders. The persistence of myalgia, loss of muscle strength and maintenance of high levels of CK, even after suspension of statins, must lead to the suspicion of an immune-mediated disorder.

P18 |

EVALUATION OF THE CONDITION UNCERTAINTY OF QUALITATIVE BINARY RESULTS AS A MEASUREMENT UNCERTAINTY ANALOGY

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Introduction: Measurement uncertainty is suited uniquely to quantitative expressions, as stated in the Guide to the Uncertainty of Measurement (GUM). However, risk-based thinking involves the determination of uncertainty of binary results (true/false, reactive/non-reactive, positive/negative). Pereira et al. (2015,2016,2019) suggest “condition uncertainty” as “the risk of false results.” Let us assume the “diagnostic accuracy” as an example of “condition uncertainty” for a more natural comprehension.

Objective: The goal is to present a novel concept of uncertainty applied to qualitative binary results.

Material and methods: The 95% confidence interval (CI) is interpreted as the interval related to the probability of an individual from 95% of the target population, such as infected individuals, being classified as positive for a specific test. The risk of this null hypothesis to be false is 0.05 or 5%. The “score confidence interval” method is recommended for the computation. Hypothetically, we will assume the validation of a new screening immunoassay to detect anti-HIV antibodies in human plasma samples in a Blood Bank - 35 infected samples, and 35 non-infected samples. The target diagnostic sensitivity (Se) is 100%, and the target diagnostic specificity (Sp) is 85%. The target of the 95% CI low limit for Se is 90% and for Sp, it is 80%.

Results: The number of true-positives is 35; false-negatives is zero, true-negatives is 34 and false-positives is one. Se of 100% where 95% of true positive results \hat{I} [90.1%, 100%]. Sp of 97.1% where 95% of true negative results \hat{I} [85.5%, 99.5%].

Discussion: Both diagnostic accuracy measurements are in accordance with what is claimed. However, the diagnostic uncertainty of Se is significantly lower (shorter interval) than the uncertainty of Sp. Therefore, diagnostic uncertainty expresses the level of confidence of the Se and Sp. On the case, the risk of false results is higher for Sp. Note that the confidence interval is not only influenced by the number of false results, but also by the number of results, n. So, lowers n cannot express lower intervals.

Conclusions: The 95% CI can be interpreted in a risk perspective as an uncertainty estimator. As the interval amplitude increases, the condition uncertainty rises accordingly, i.e., the risk to classify false results increases. The interpretation is, to some degree, similar to what happens with the expanded measurement uncertainty.

P19 |

TRADITIONAL VS. REVERSE ALGORITHM IN SYPHILIS SEROLOGICAL DIAGNOSIS: RETROSPECTIVE STUDY IN DISTRICT HOSPITAL

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Introduction: Serological testing is regarded as the usual diagnostics for syphilis. Currently, traditional and reverse algorithms are used to its performing. Traditional algorithm initiates with a non-treponemal test, followed by treponemal test for the confirmation. In the reverse algorithm the treponemal immunoassay is used for reactivity screening with subsequent non-treponemal test realization.

Both algorithms have several limitations, accuracy wise and uncertain interpretations by clinicians, which inevitably leads to controversies in syphilis diagnosis.

Description: We performed a retrospective study of syphilis diagnostic algorithm shift that occurred in our Laboratory by evaluating traditional algorithm positive results obtained between January 2014 and December 2015 and reverse algorithm positive results from January 2017 to December 2018.

In the 1st period of time, 5782 Rapid Plasma Reagin (RPR) determinations were executed with 113 (1.95 %) positive samples, confirmed by *Treponema pallidum* hemagglutination assay (TPHA) or fluorescent treponemal antibody absorption test (FTA-abs), and 19 (0.33 %) false positive results.

In the 2nd period, we performed 5960 treponemal quimioluminescent immunoassays (CLIA) with 323 (5,42%) positive results, confirmed by TPHA, and just 146 (2.45%) were reactive to RPR. Therefore, 177 (2.97%) samples remained negative if tested by the traditional algorithm. The missed diagnosis rate was 54.8%. Using reverse algorithm only 19 (0.32%) of treponemal CLIA determinations were false positive.

Discussion: There have been important advancements in the serologic diagnostic tests for syphilis in the past years. Traditional syphilis algorithm remains in use, but the reverse algorithm has stepped out the leading diagnostic tool.

Considering that RPR interpretation is subjective and depends on staff experience, the shift to a CLIA treponemal automatic and standardized assay improved the outcome.

Diverse studies suggest that the results of RPR screening may correlate well with disease activity however being concerning that they may miss the diagnosis of late latent and early primary syphilis.

Considering numerous recommendations, we routinely apply reverse syphilis diagnostic algorithm in our laboratory, which has increased the detection rate.

P20 |

ADALIMUMAB'S THERAPEUTIC DRUG MONITORING – IS FASTER ALSO BETTER? – A COMPARATIVE STUDY BETWEEN TWO METHODS

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Introduction: Biological drugs are widely used in the treatment of immune-mediated diseases. Tumor necrosis factor-antagonists such as adalimumab (ADM) are first-line therapy in controlling the underlying inflammatory process and are associated with high rates of clinical and laboratory remission. Yet some patients present with primary or secondary response failure to the immunosuppressant which can be caused by pharmacokinetic or pharmacodynamic variations or by its immunogenicity. The drug's efficiency is associated with its serum levels which is why therapeutic drug monitoring (TDM) has been used as a tool in therapy optimization. The standard laboratory method for performing TDM is the enzyme-linked immunosorbent assay (ELISA) however it can take several hours before results are available. Therefore, point-of-care (POC) solutions have arisen using different methods such as lateral flow immunochromatography which can deliver results in few minutes.

Objective: To compare two different methods used for ADM's TDM and determine whether they are interchangeable.

Materials and Methods: A RIDA® QUICK ADM Monitoring kit (25 determinations) was used in the POC solution RIDA® QUICK SCAN II with selected serum samples that were previously analyzed by ELISA with the LISA-TRACKER Duo ADM kit on the Dynex® DS2 analytical system. 19 valid results (n = 19) were obtained from which Pearson's correlation coefficient and linear regression were determined.

Results: Pearson's correlation coefficient was 0.95. The linear regression was $R^2 = 0.91$ for a $y = 1.311x + 1.238$.

Discussion: There is a strong positive correlation between both methods used in the ADM's TDM however it isn't possible to do statistically significant conclusions due to the sample size. If with a larger sample the same correlation is observed then is possible to conclude that both methods are interchangeable. In theory this could mean that ADM's serum levels can be available to the clinician much faster allowing a better treatment management, yet in practice it'd require a technician to be assigned to process the samples and handle the equipment whenever there's a TDM request which isn't tangible in a laboratory routine with a high workload. Therefore POC can't replace the standard method but can be used as a viable option whenever a quick assessment is needed.

P21 |

A PRACTICAL APPROACH TO EFLM CONSENSUS ON IMPLEMENTATION OF METROLOGICAL TRACEABILITY AS INTENDED BY ISO 15189 FOR LABORATORY HARMONIZATION

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Introduction: In a previous work (1) we used peer reports (PR) of commercial controls to define the combined measurement uncertainty (COU) for 4 clinical chemistry analyzers of our 3 hospitals, that gives the same reference change value, and thus harmonize results (HR) as if there is only one analyzer.

In 2019 the EFLM Working Group Accreditation, issued a consensus statement for the Implementation and documenting metrological traceability (MT) as intended by ISO15189.

We show a practical approach, using PR, intraindividual biological variation (CVi) and uncertainty of measurement (UM). following the European Research Centre for Metrological Traceability in Laboratory Medicine (CIRME) recommendations to detect commercial calibrators (CC) suitable for clinically safe HR, and document MT.

Material and Methods: UM cannot be eliminated and all single uncertainty contributions across the MT chain add up to obtain the COU associated with results for clinical samples at the bottom of the chain, for the HR.

In 2019 CIRME stated that the CC should have 50% of a total budget of COU (GU), that is confined within an established target, making suitable the measurement in the clinical field. The other 50% of GU is assigned to the laboratories to spend that uncertainty budget for the random sources.

On our previous works we concluded that when the expanded allowable UM (APS) is the same as CVi, it maintains the predefined clinical safety set of results, because all errors within those APS do not affect the patients results distribution charts (PDC) used to monitor the analytical stability. (2,3)

The expanded UM is 2xCV. When 2xCV=CVi as the GU, obtained from EFLM Biological Variation database (BV), that means we are using the defined 50% of GU for clinically safe HR's MT, following CIRME.

With use MULTIQUAL lot 45800 BioRAD for PR.

Results: PR shows we can set an APS with CV for CK= 4%, Urea=6%, K= 1.5% and PCR=7%. BV CVi data is for CK= 16%, Urea=13.9%, K= 4.1% and PCR=33.5%.

We have a CV under 50% of CVi on those magnitudes.

Conclusion: Using expanded UM as our APS, we have the internal quality control as a MT documentation. Using PR and BV we can spot those CC with UM within 50% of CVi, and safe for clinical use in HR

P22 |

COMPARISON OF AN AUTOMATED METHOD WITH A RAPID INFLIXIMAB TEST IN THERAPEUTIC DRUG MONITORING OF PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Introduction: Biological drug therapy is being increasingly used. Drugs with different mechanisms of action are currently available, with tumor necrosis factor (TNF) antagonists, such as Infliximab (IFX) being the most widely used. Infliximab (IFX) is a chimeric human-murine antibody used in the treatment of various autoimmune, chronic inflammatory and rheumatological diseases. There is a significant percentage of patients who do not respond adequately to therapy. The reason for therapeutic failure is not always clear and may involve rapid drug elimination, inadequate dosing or issues of immunogenicity and bioavailability. Currently, therapeutic monitoring (TDM) of IFX is proposed to provide clinicians with useful information as a way to improve treatment effectiveness. Since ELISA techniques have a longer laboratory turnaround time, we intend to evaluate the feasibility of using a point of care method for a faster response to clinical needs. Differences in analytical sensitivity and specificity between different test platforms can lead to discrepancies in the measured serum concentrations.

Objective: This study aimed to analyze the data obtained in different tests in patients undergoing IFX therapy in order to assess the possible interchangeability of methods.

Materials And Methods: We tested 18 serum samples from IBD patients from the Gastroenterology Service of a university hospital. An automated IFX ELISA kit (Theradiag Lisa-Tracker Duo) was used in the Dynex® DS2 system and a point-of-care system with lateral flow immunoassay (Bühlmann Quantum Blue®). The data obtained were treated and compared statistically through the application of linear regression and Pearson's correlation coefficient (ρ).

Results: The results obtained by ELISA showed a mean of 5.03 $\mu\text{g/mL}$ and a median of 5.56 $\mu\text{g/mL}$ and by the point-of-care 8.41 $\mu\text{g/mL}$ and 8.95 $\mu\text{g/mL}$, respectively. Pearson's coefficient was 0.87 for weighted linear regression ($y = 1.665x + 0.0418$).

Conclusion: The evaluated data allow us to conclude that both methods are feasible for IFX assay but given the correlation coefficient and the small size of the studied sample, the interchangeability of the methods must be carefully considered.

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PREANALYTICAL SAMPLE HANDLING IN EUROPE AND PORTUGAL... ALL WE NEED IS CARE!

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Introduction: Compared to other activities of the testing process, the pre-analytical phase (PP) is plagued by a lower degree of standardization, which makes it more vulnerable to errors. In the last decade, pre-analytical issues (PI) have been addressed more extensively, with dedicated working groups being developed throughout Europe.

Aim: An overview of the European Federation of Clinical Chemistry and Laboratory Medicine Working Group for the Pre-analytical Phase (EFLM WG-PRE) survey across European medical laboratories including Portugal, to gather information on local pre-analytical practice.

Materials and Methods: Based on a review of a coherent article of the EFLM WG-PRE, which covered all practices on monitoring pre-analytical quality except haemolysis, icterus and lipemia. An online survey containing 39 questions dealing with a broad spectrum of PI, was disseminated to EFLM member countries, included questions on willingness of laboratories to engage in PI.

Results: Of the 37 countries which completed the survey, valid responses were received from a total of 1.347 participants. From these, Portugal was one of the most frequent responder with 61 (4.5%) participants. Ninety four percent (N=1265) stated they monitored/documented pre-analytical errors (PE), with Portugal featuring many laboratories reporting to monitor those. Of 875 laboratories which did evaluate data from PE, further use of this information varied among respondents and countries. A total percentage of 93% (N = 1255) of responders analysing blood samples stated that they provided pre-analytical guidance on laboratory parameters to the sender in many ways. Interest of participants in PI and their willingness to actively engage in this topic varied. The majority of responders stated to be interested in a guideline for the measurement and evaluation of pre-analytical variables (92%; N =1235). Stability of analytes, analytical interference and compliance to venous specimen collection guidelines was an expressed concern.

Discussion: Monitoring the PP of the total testing process and acting upon it varies largely throughout Europe. The interest in PI is pleasantly high. This important data will be used by the WG-PRE for providing recommendations on the most critical issues.

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DERMATOMYOSITIS WITH ANTI-SAE ANTIBODIES - CLINICAL CASE

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Introduction: Anti small ubiquitin-like modifier activating enzymes (SAE) antibodies are specific of myositis and have an estimated prevalence of 5-8% in the European population with dermatomyositis. These antibodies are clinically associated with systemic symptoms, severe skin disease and dysphagia. Regarding interstitial lung disease, the clinical presentation is generally mild despite imaging changes.

Clinical Case: Female, 71 years old, was admitted for investigation of probable overlapping syndrome of Sjögren's syndrome and dermatomyositis. With symptoms of xerostomia, xerophthalmia and photosensitivity framed in Sjogren's syndrome and non-heliotropic facial erythema, cutaneous lesions compatible with Gottron's papules, loss of proximal muscle strength, myalgia and high dysphagia for solids, suggestive of overlap with dermatomyositis. The patient had radiological changes suggestive of interstitial lung disease, still under study. Analytically, presented anti-nuclear antibodies with an immunofluorescence pattern, according to the ICAP classification, AC-4 (Nuclear fine speckled) with titre >1280 identified as anti-SSA. For the study of dermatomyositis, antibodies specific to myositis were requested, which revealed anti-SAE antibodies. Clinically stable, the patient was discharged with the indication for a follow-up appointment with an electromyography and muscle biopsy to complement the study.

Conclusion: Clinically, the patient had severe skin disease and dysphagia, symptoms frequently associated with the presence of anti-SAE antibodies. Although not yet possible to assess due to its recent diagnosis, it has been proved the existence of an association between the positive response to immunosuppressive therapy and consequent favorable prognosis associated with the presence of this autoantibody.

The study of specific antibodies associated with myositis is paramount to the diagnosis, prognosis and therapeutic guidance in dermatomyositis, due to the association of these antibodies with clinical characteristics, response to therapy and prognosis.

P25 |

SERUM INDICES QUALITY CONTROL – A WORTHY ALLY IN THE AUTOMATED LABORATORY

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Introduction: For years visual, inspection would help identify optical interference in chemistry samples. With the advances in automation, systematic assessment of plasma and serum samples for haemolysis (H), icterus (I) and lipemia (L) became routine practice, having a direct impact on post-analytcs.

Aim: We aimed to verify whether the Liquicheck™ Serum Indices kit (Bio-Rad Laboratories) is suitable for quality assurance of HIL indices in a central hospital with a laboratory automation track system.

Material and methods: The Liquicheck™ Serum Indices kit includes 4 liquid samples (4 mL) derived from human-sourced material, with a 14 day open-vial stability (2–8°C). Included are 1 level for each serum index in different vials and 1 non-interfered one.

Samples were manually programmed in each of our 8 Alinity c modules (Abbott) and measured as routine samples, following the manufacturer's recommendations.

The H, I and L indices were reported in arbitrary units convertible into concentration of haemoglobin, bilirubin and triglycerides.

All results were recorded, compared with the provided reference values and further analysed with Microsoft Excel.

Results: The samples were analysed on days 0, 4 and 9 of storage after thawing (n=303). The intra-assay imprecision of HIL indices was calculated by measuring the fresh materials in a single analyser for 6 consecutive runs, with excellent reproducibility (H: x=125.5, SD=1.1; I: x=20.2, SD=0.37; L: x=361.8, SD=4.3). There was very good concordance between analysers (H: x=121.8, SD=6.5; I: x=17.1, SD=0.8; L: x=298.1, SD=67.0) despite some variation of interfering substances with time (-9%, -7% and -36% for H, I and L, respectively).

No measurement exceeded the performance goal of 3SD calculated for each quality control material.

Conclusion: The Liquicheck™ Serum Indices kit is helpful in the validation of the performance of HIL indices. In spite of the inconvenience of having to manually programme the samples, the implementation of a quality control system for HIL assessment in a central hospital with a laboratory automation track system is advantageous, as the visual check is hardly feasible for confirming unexpected findings. Routine assessment of HIL indices has been endorsed by CLSI and EFLM, and will inevitably soon be generalised in laboratories worldwide.

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ASSESSMENT OF PLASMATIC STABILITY OF ISONIAZID - A CONTRIBUTION TO THE BEST PRACTICES FOR TDM

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Introduction: Isoniazid (IZD) is a first line tuberculostatic drug with assumed advantage in clinical outcome by the individually adjusting of the dose. Hepatic metabolization is determined genetically by acetylation phenotype precipitating high pharmacokinetic variability. Therefore, determination of plasma concentrations disclosures essential in the treatment success. Frozen conditions of the samples are widely used to minimize IZD degradation and the samples are often maintained at -80°C temperatures but, before storage, the assessment of the pre-analytic stability is crucial for laboratories to define internal procedures for sample preparation.

Aim: The aim of this study is to evaluate the IZD degradation by temperature exposure since the arrival of samples until storage, in order to define the appropriate sample flow in the laboratory in best TDM practices.

Material and methods: Four plasma samples were received and centrifuged in a refrigerated way. An aliquote was made and stored at -80°C (T0). The remain sample was left at room temperature (RT) and the same aliquoting proceeding was performed in the defined times: after 30 minutes (T1), 60' (T2), 90' (T3), 120' (T4), 180' (T5) and 240' (T6). IZD was quantified (mg/L) using a LC-MS/MS method in a single analytical run. Result bias (%) were calculated.

Results: Mean IZD bias variation was: -7,2% in T1 (+- 4,3 SD), -10,3% in T2 (+- 9,8 SD), -8,7% in T3 (+- 1,9 SD), -7,9% in T4 (+- 9,3 SD), -1,9% in T5 (+- 13,5 SD), and -9,8% in T6 (+- 27,9 SD), compared to T0.

Conclusions: Despite the few samples in this study, the high bias variability in T6 discourage RT for 4 hours. Accepting the fact that a result variation less than 10% (bias) is consistent with the measuring methodology, a 3 hours circuit is suitable for laboratories workflow, assuring best practices for isoniazid TDM.

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SEVERE IATROGENIC HYPERTRIGLYCERIDEMIA IN ACUTE PROMYELOCYTIC LEUKEMIA – THE IMPORTANCE OF LIPID LEVELS MONITORING

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Introduction: All-trans-retinoic acid (ATRA) has been successfully used in the treatment of acute promyelocytic leukemia (APL). Hypertriglyceridemia (HTG) is one of the most frequently observed systemic effects of retinoid therapy.

Case report: A 17 year old boy, with late molecular relapse APL (>36 months after diagnosis) presented with HTG. The patient has started treatment with ATRA, arsenic trioxide and gemtuzumab ozogamicin. Complete remission was achieved after induction and consolidation therapy was initiated. During the whole course of treatment, the patient revealed sustained and severe HTG (> 1000 mg/dl). After the 4th ATRA consolidation cycle (6 months of treatment) the patient achieved a maximum level of triglycerides of 4344 mg/dl and 656 mg/dl of cholesterol. Other than a mild increase in serum lipase 79,0 U/L, aspartate transaminase 71 U/L, alkaline phosphatase 131 U/L and lactate dehydrogenase 358 U/L, the remaining biochemistry findings were within reference range, namely amylase, alanine transaminase, gamma-glutamyl transpeptidase and bilirubin levels. The patient was clinically well, with no evidence of acute illness. Considering the clinical-laboratorial context, the treatment was not suspended, and the patient remained under surveillance.

Discussion: Although not fully understood, retinoids may cause hyperlipidemia by interfering with lipid clearance. Studies suggest the existence of a genetic predisposition for an “overreaction” to vitamin A derivatives, which can become evident during therapy and let a masked lipid metabolism disorder become manifest.

HTG can occur during ATRA treatment, albeit rarely requiring an adjustment in treatment. Severe HTG (>1000 mg/dl), a significant risk factor for acute pancreatitis, is a rare but serious adverse event of systemic therapy with these drugs, thus the importance of serum lipid levels and pancreatic function monitoring in these patients.

According to literature, there is also an increased atherogenic risk due to prolonged dyslipidemia/hyperlipidemia during long-term use (> 6 months) of vitamin A derivatives.

After discontinuation of therapy, serum lipid levels should be evaluated, in order to confirm that changes were temporary or to exclude a further existing disorder of lipid metabolism with no association to retinoids.

IS A POSITIVE FECAL IMMUNOCHEMICAL TEST FOR HAEMOGLOBIN SYNONYM OF ANEMIA?

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Material and Methods: In this retrospective study all FIT samples and hemogram from 8111 patients of the District of Aveiro, in ambulatory regime, collected at the Clinical Analysis Laboratory Avelab during the period 1 January 2019 and 31 December 2019 were analyzed. The study was approved by the Ethical Committee of the Clinical Analysis Laboratory Avelab by Doctor Américo Freitas. In the pre-analytical phase patients were instructed how to collect a fecal sample according specific instruction. The fecal material was collected in a sampling tube and analyzed (SentiFIT® 270). Each patient collected three sampling tube. The cut-off value applied was 50 ng/mL of hemoglobin (Hg). It was established < 50 ng/ml of Hg as a negative FIT and ≥ 50 ng/mL of Hg as a positive FIT. The primary end-point was to find the % of total positive FIT and the % of positive FIT for each gender. The secondary endpoint was to evaluate the influence of a positive FIT in the value of [Hg]. The collection of venous blood sampling was performed by venipuncture (BD Vacutainer® spray-coated K2EDTA). The Hg determination was analyzed using Sulfolyser Hematology Solution (Sysmex XE-2100). The cut-offs applied for determination of anemia were ≤12,0 g/L of Hg for women and ≤13,0 g/L of Hg for men. Excluding criteria in the study were patients aged 0 – 18 years old. The data were treated using the Excel® for Windows Vista®. The normality of data, homogeneity and independence of variance were checked before analysis. Continuous variables were reported as mean with standard derivation. Qualitative variables were expressed as frequencies and percentages. For each gender it was applied an ANOVA (p < 0,05). **RESULTS:** A total of 8111 patients were included in the study, 6414 (79%) in the negative FIT group and 1697 (21%) in the positive FIT group. The mean age of the patients was 63 ± 11 years old. In the positive FIT group we obtained a distribution of 779 (46%) women and 918 (54%) men. In the positive FIT group, 8% (n = 74) men and 10% (n = 78) women have anemia. **CONCLUSION:** A positive FIT is not directly associated with anemia in both genders. The cut-off applied is very low to reflect an effective decreased of values of Hg. However, this cut-off is adapted to local screening colorectal program, to obtain an earlier detection of colorectal cancer, in ambulatory regime.

TUMOR MARKERS IN ABDOMINAL AND PELVIC TUMOURS- MYTHS AND FACTS UNFOLDED

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Introduction: The majority of Tumor Markers (TM) are blood-soluble glycoproteins produced by the body, in the presence of malignancy and can be found in many tissues and body fluids. TM plays an important role in cancer detection and management. Each TM has a variable profile and is useful for screening, determining diagnosis and prognosis, assessing response to therapy, etc. TM can be elevated and specific in the setting of cancer but, they may be present in different types of cancers, may not show marker elevation and, benign conditions can cause false-positive elevation. Commonly used TM in abdominal and pelvic tumours (APT) include carcinoembryonic antigen (CEA), total prostate-specific antigen (T-PSA), alpha-fetoprotein (AFP), carbohydrate antigen 19.9 (CA-19.9), carbohydrate antigen 125 (CA-125), the novel human epididymis protein 4 (HE4).

Aim: To describe the commonly used TM in APT from 01/01/2017 to 31/12/2019 in a tertiary referral hospital, their limitations and use in the context of known cancer.

Methods: Data search by Clinidata XXI® Software (Maxdata). Method used to determine the TM- Electrochemiluminescence (ECLIA).

Results: A total of 159.410 TM were requested. CEA (47.763-30%): most widely used for colorretal carcinoma. For use in Staging (S), Surgical Planning (SP), Treatment Monitoring (TM) and Prognosis (P). AFP (25.746-16%): used for hepatocellular and testicular carcinoma. For use in (S), (TM), (P). CA 19.9 (25.689-16%): most widely used for pancreatic cancer. For use in (S), (SP), (TM), (P). CA-125 (5.563-3.5%): widely used for epithelial ovarian malignancy (EOM). For use in (S), (SP), (TM), (P). HE4 (169-0.5%): EOM. Higher sensitivity than CA-125. Used as part of the ROMA (Risk of Ovarian Malignancy Algorithm) to stratify the risk for EOM in pre- and pos-menopausal women. The above are not for use as a Diagnostic Test (DT) or Screening Test (ST). T-PSA (54.480-34%): prostate cancer. For use in (S), (SP), (TM), (P) and (ST) in particular settings.

Conclusion: TM values are an important diagnostic tool. However, these have limitations (mainly lacking diagnostic specificity) and should not be used solely, but interpreted in conjunction with diagnostic imaging, clinical history and physical examination that will help optimize the multidisciplinary care and management of oncologic patients.

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DIAGNOSING DISSEMINATED HISTOPLASMOSIS IN AN AIDS PATIENT- THE ROLE OF BONE MARROW EVALUATION

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Background: Histoplasmosis (HP) is a mycosis resulting from the inhalation of spores from *Histoplasma capsulatum*. Cases are most described in the Americas and Africa. *H. capsulatum* is a dimorphic fungus with a mycelial and a yeast phase. In the immunocompromised host it is opportunistic and can invade the bloodstream and spread to other organs, causing Progressive Disseminated Histoplasmosis (PDH), a potentially fatal disease. There are multiple methods to diagnose HP, but the gold standard for identification is the culture demonstrating the thermal dimorphism of the fungus.

Aim: to present a case of PDH in an AIDS patient through Bone Marrow (BM) evaluation.

Materials/methods and results: A 52 year old male, with treated HCV, HIV-1 infection (CDC-C3, CD4+: 4,5%, 27 cel/ μ L, HIV-RNA: 5640000 copies/mL) and a recent trip to Brasil, referred to the ICU of our hospital with long term fever, astenia, caquexia and recent episodes of haemoptysis and scattered skin bruises. A RT-PCR of BAL was positive for *Pneumocystis jirovecii*. Laboratory analysis were consistent with haemophagocytic lymphohistiocytosis (96 to 98% probability score). A BM aspirate films stained with May-Grunwald-Giemsa revealed: yeast-like bodies inside and outside macrophages with morphology suggestive of *Histoplasma* spp. BM specimen was cultured in duplicate on Columbia blood agar, Sabouraud dextrose agar (SDA), SDA with gentamicin/chloramphenicol, SDA with cicloheximide and incubated at two temperatures (25°C and 37°C). After 7 days: at 35°C- growth of white, smooth, creamy and yeast-like colonies. Microscopic examination of a Lactophenol Blue Mount Preparation (LBMP): round to oval, small, budding, and thin-walled yeast-like cells. At 25°C- growth of velvety/cottony white colonies with yellow reverse. LBMP: hyphae hialine and septate, with short conidiophores. Macroconidia were large, round, one-celled, hyaline, thick-walled and tuberculate (features consistent with *Histoplasma capsulatum*). Molecular results (BM PCR) revealed- *Histoplasma capsulatum*.

Conclusions: PDH carries a poor prognosis. Giving its rarity it poses a real challenge but should be considered, specially in AIDS patients. In our case the diagnosis was possible through microscopy and culture (sensitivity of BM culture is 75%).

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STREPTOCOCCUS SUIS MENINGITIS - CASE REPORT

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Introduction: *Streptococcus suis* is a gram-positive, encapsulated bacterium that colonizes the respiratory and digestive tracts of swine. It can be transmitted to humans through contact with infected animals or consumption of contaminated meat. It can cause septicemia, pneumonia, endocarditis and meningitis and it can cause irreversible sequelae such as hearing loss.

Case description: 57-year-old man, pig farming, reports fatigue, myalgia and left omalgia after roasting piglets. After 3 days, he starts headache and fever, with changes in behavior, followed by prostration. In the emergency department, he presents prostrate, with stiff neck and a temperature of 38.8°C. Cranial CT-scan was performed: no abnormalities were found. In the analytical study: Leukocytosis of 11310 / uL with neutrophilia of 83.9% and CRP 232.4 mg/L.

Lumbar puncture was performed: cloudy Cerebrospinal fluid (CSF), 7059 cells with 91% neutrophils, 70 erythrocytes, glucose <4 mg/L, proteins 4.57 g/L. Gram-positive cocci in chains were observed. A Meningitis / Encephalitis Panel (Biofire® Filmarray®) was performed, being negative for all tested microorganisms. Three blood cultures (BC) were taken. They were all positive in less than 24 hours, with gram positive cocci. Antigen test for Pneumococcus was negative.

The following day, a strain of *Streptococcus suis* (MALDI-TOF MS - Bruker®) was identified in BC and CSF. It was resistant to clindamycin and erythromycin and sensitive to penicillin, ampicillin, cefotaxime (BioRad® discs) and vancomycin (E-TEST bioMerieux®).

After 21 days of hospitalization, the patient develops sudden left hypoacusis, assuming a likely late cochlear / labyrinthitis as a result of meningitis.

The patient completed 21 days of ceftriaxone and one cycle of dexamethasone.

Discussion: This clinical case describes the common clinical presentation and the sequelae associated with *Streptococcus suis* infection.

The infection by this agent is potentially fatal, if not treated in a timely manner and should be considered in cases of meningitis or septicemia and a compatible epidemiological context. Infection control measures must be taken with infected animals.

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EVOLUTION OF THE SUSCEPTIBILITY PROFILE OF CAMPYLOBACTER SPECIES ISOLATED AT THE CENTRO HOSPITALAR E UNIVERSITÁRIO DO PORTO

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Introduction: Campylobacter infection is the main cause of acute bacterial gastroenteritis worldwide. The first line of therapy includes macrolides or quinolones. Alternatively, tetracycline can be used. Several studies have shown that species isolated from stool samples are often resistant to antimicrobials used as the first therapeutic line.

Objectives: To determine the rate of resistance to antimicrobials of Campylobacter species isolated in stool samples at the Microbiology Service of CHP, from early 2017 to early 2019, and to compare the results of this investigation with the data obtained in a previous study from early 2013 to early 2015.

Material and methods: 50 strains of Campylobacter spp isolated from stool samples were identified by Gram method, catalase and oxidase tests and by the automatic system Vitek2® and VitekMS®. The susceptibility assessment was performed by diffusion method on agar with ciprofloxacin (26mg), erythromycin (20mg) and tetracycline (30mg) discs on Mueller-Hinton F® medium, according to the EUCAST recommendations. Quality was ensured by using the Campylobacter jejuni strain ATCC (33560). The data were processed by using the SPSS® v20.0 program and a t-student test for paired samples.

Results: Of the 50 samples studied, 92% were Campylobacter jejuni and 8% Campylobacter coli. In the current study C. jejuni had a resistance rate of 84.2% to ciprofloxacin, 69.6% to tetracycline and 8.6% to erythromycin. In the previous study (2013-2015), C. jejuni had a resistance rate to ciprofloxacin of 88.2%, tetracycline of 62.7% and erythromycin of 3.9%. The comparison of samples by the t-student test (CI>95% and P-value<0.05) revealed no statistically significant changes between the susceptibility profile for the two distinct periods of time. All strains of C. coli were resistant to ciprofloxacin and tetracycline and half were resistant to erythromycin.

Discussion: There were no significant changes in the susceptibility profile to ciprofloxacin, tetracycline and erythromycin. Therefore, a pattern of high resistance to ciprofloxacin and tetracycline was observed, on the other hand, the high susceptibility to erythromycin was maintained. This study proved that the best option for empirical treatment would be the administration of a macrolide, in this case erythromycin.

SPONTANEOUS BACTERIAL PERITONITIS DUE TO PASTEURELLA MULTOCIDA – A CASE REPORT

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Introduction: Spontaneous Bacterial Peritonitis (SBP) can be a life-threatening complication of cirrhosis and is usually caused by enteric Gram-negative bacteria (GNB). *Pasteurella multocida* (Pm), a zoonotic GNB, has been rarely associated with peritonitis in humans. It has a high mortality rate, despite appropriate treatment.

Case Description: This is a case report of a 56-year-old male admitted to hospital with progressive abdominal distension, dyspnea, fatigue and oliguria in the two weeks prior to the admission. The patient also described a cat bite in the previous days. He had a history of alcoholic cirrhosis and was hypocoagulated due to portal vein thrombosis.

The patient was hypotensive, and the blood gas analysis revealed hyperlactacidemia, hypoxemia and hypoglycaemia. He had peripheral edema and a distended abdomen due to ascites - a diagnostic paracentesis was performed and fulfilled the criteria for SBP.

On physical examination, it was noted generalized cutaneous fragility and a bite wound in the forearm.

Antimicrobial therapy with Cefotaxime was initiated after blood and ascitic fluid were drawn for culture. Blood cultures were negative.

Inoculation of a blood culture bottle with ascitic fluid in aerobic conditions yielded a GNB that grew on chocolate agar but not on MacConkey agar. Matrix Assisted Laser Desorption/Ionization Mass Spectrometry analysis identified the agent as Pm.

Antimicrobial susceptibility testing demonstrated it to be susceptible to Cefotaxime.

His condition progressively deteriorated requiring admission to an Intensive Care Unit and died after seven days of hospitalisation with multiple organ dysfunction syndrome.

Discussion: Pm is usually transmitted by canines and felines. Cases of peritonitis associated with Pm have been reported in patients with hepatic cirrhosis and in patients on peritoneal dialysis. It can colonize the respiratory and gastrointestinal tracts. In addition to infection through skin lesions, there have also been reported unknown ways of transmission in several human infections.

Conclusion: Because hepatic cirrhosis leads to immunological defects, these patients need to be careful when handling canines and felines. This case illustrates a rare cause of SBP that is associated with high mortality.

URINE CULTURE: COSTS OF PREANALYTICAL ERRORS

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Introduction: Pre-analytical errors account for 46-68.2% of errors observed during the Total Testing Process, most of which arise from problems in patient preparation, sample collection, transportation, and preparation for analysis and storage.

Urinalysis is the third major diagnostic screening test in the clinical laboratory, preceded by serum/plasma chemistry profiles and complete blood count analysis. Well-standardized procedures for collection, transport, sample preparation and analysis should become the basis of an effective diagnostic strategy for urinalysis. It is mandatory to focus on the preanalytical phase in order to lower the costs of health care.

But how much cost a preanalytical error in the laboratory of microbiology?

Objectives: Quantify the number of urine samples with mixed growth indicative of contamination. Estimate the cost associated with the processing of these samples in the laboratory. To determine the relevance of defining awareness programs to reduce the number of samples improperly collected or transported.

Materials and methods: A total of 116103 urine culture requisitions (data from years: 2017, 2018 and 2019) were retrospectively analyzed, quantifying urine samples with mixed growth indicative of contamination. The cost associated with the processing of each of the contaminated samples was estimated, considering the costs with human resources, materials and equipments used and residues disposal. The total cost associated with the processing of these samples was determined.

Results: Of the 116103 urine culture requisitions, 9696 were considered contaminated. The costs associated with laboratory processing of each sample were estimated in 1,98 euros, which corresponded to a total of 19198 euros in the total of 9696 contaminated samples.

Conclusion: From the analysis of these results, we can conclude that, taking into account the costs associated with the processing of contaminated samples, it is absolutely advantageous to develop an awareness program to reduce the number of contaminated urine samples. This particular example, emphasize the importance of the preanalytical phase in the management of the laboratory and development programs that reduce preanalytical errors in the laboratory.

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RETROSPECTIVE STUDY OF SEXUALLY TRANSMITTED DISEASES IN CENTRO HOSPITALAR UNIVERSITARIO SÃO JOÃO

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Introduction: Sexually transmitted diseases (STDs) have long been recognized as a major public health problem all over the world. The disease burden of STIs globally is unknown; as asymptomatic infections are frequent, diagnostic techniques are not always available.

Objective: We conducted a retrospective observational study from biological samples recollected at Centro Hospitalar Univesitário São João (CHUSJ) (Porto). The infectious diseases assessed in this study were Chlamydia trachomatis, Neisseria gonorrhoea, Treponema pallidum Trichomonas vaginalis, Mycoplasma genitalium

Methods: Data were collected between 2016 to September 2019 from Clinidata[®] XII and analyzed using exploratory and descriptive statistics using the SPSS version 25. All microorganisms were detected using molecular tools like Real Time PCR and Multiplex PCR.

Results: A total of 13909 samples for STDs researched from January 2016 to September 2019 were registered. N. gonorrhoea represents 42.6% of the requests followed by C. trachomatis with 32.8%. T. pallidum and T. vaginalis represent 4.3% and 8% respectively. During 2016 to 2019 the rate of positivity for N. gonorrhoea and C. trachomatis ranged between 3.22%-6.69% and 4.76%-8.77% respectively. Regarding biological samples, the majority of the isolates for N. gonorrhoea and C. trachomatis came from urethral exudate and urine samples. For T. pallidum 166 isolates were detected in cerebrospinal fluid samples, representing 27,6% of all requests for this microorganism. M. genitalium was requested in 12.1% of overall samples, with a positivity rate of 4.3%, mostly from rectal exudates in males.

Conclusion: The pattern of STDs varies in different places. Gonococcal infections are still the commonest STD seen in our patients, followed by C. trachomatis. The emergence of M. genitalium in males in the last years became a public health problem.

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NOCARDIOSIS - A CLINICAL CASE

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Introduction: Nocardiosis is an infection caused by actinomycetes in the genus *Nocardia* and can cause localized or systemic disease. It is a rare but severe disease associated with high mortality, typically considered an opportunistic agent, causing disease in immunodepressed patients. Risk factors associated with *Nocardia* spp. infection include glucocorticoid therapy, malignancy, organ and hematopoietic stem cell transplantation, and Human Immunodeficiency Virus infection. Solid tumors and hematologic malignancies are responsible for a large part of nocardiosis.

Case: Male, 73 years, retired from the metallurgical industry. History of hydrocele and prostate cancer. Followed in the Hematology consultation for Non-Hodking B Lymphoma under treatment. During a chemotherapy cycle, he had fever of 38.1°C. The treatment was interrupted, he took antipyretic and were collected blood cultures. In the next day, he performed a control computed axial tomography scan, recommended in these treatments, with images suggestive of liver abscess. Subjected to ecoguided drainage with collection of fluid from the liver abscess for microbiological study. Admitted to the General Surgery Service, empirically medicated with piperacillin / tazobactam, with favorable evolution. In the microbiological examination of liver abscess exudate was isolated a Gram-positive, branched, bacteria compatible with *Nocardia* spp. Positive blood cultures with the same agent.

Discussion: *Nocardia* spp are found in the environment, but rarely causes disease in immunocompetent individuals. This case presents an immunocompromised patient, with hematological neoplasia being treated, with bacteremia due to *Nocardia* spp. These patients depend on vascular access devices, which may be the predisposing factor for cases of bacteremia, as reported in the literature. This agent has a slow growth, so it needs a prolonged incubation, which may explain some difficulties in its isolation if the cultures are discarded earlier. For that very reason, it is very important that the laboratory is alerted by medical to this diagnostic hypothesis.

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LATENT TUBERCULOSIS INFECTION IN HEALTHCARE PROFESSIONALS OF A DISTRICT HOSPITAL IN CENTRAL PORTUGAL

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Introduction: Tuberculosis (TB) is an infectious disease caused by *M. tuberculosis*. There are two main categories of tuberculosis infection: active disease and latent infection (LTB), the latter being an asymptomatic, non-infectious state with potential to progress to active disease. In low to intermediate-incidence countries, such as Portugal, screening and treatment of LTB are the pillars of TB eradication.

Healthcare professionals (HP) are a high-risk group for contracting TB and literature suggests a higher incidence (25-33,2%) of LTB in HP than in general population. Consequently, Portuguese Health Directorate General recommends periodic screening for LTB in HP through currently available tests, such as interferon-gamma release assay (IGRA).

Objective: To perform the epidemiological evaluation of LTB in HP of a Portuguese district hospital.

Material and Methods: A retrospective study that included 1383 HP screened for LTB using IGRA test (QuantiFERON®-TB Gold) taken from January 2017 to December 2019. Data regarding IGRA test results, gender, age, professional group and work department were collected using Modulab®. Statistical analysis was performed using Spearman rank correlation tests with IMB® SPSS.

Results: Data from 1383 HP, with a mean age of 43.5±11.3 y.o., were analyzed. IGRA was positive in 14% of HP and indeterminate/dubious in 6,1%, imposing a second determination that was positive in 13,1% of cases. The professional group with the highest prevalence of positive IGRA were Clinical Scientists and Pharmacists (26,3%). Clinical departments presented the highest prevalence of positive IGRA (17,8%). A small but significant positive correlation (ρ 0,17; $p < 0.01$) between age and positive IGRA test results was found. No correlation between positive IGRA and professional group or work department was found.

Discussion / Conclusion: The obtained data revealed a lower LTB prevalence in HP than that found in literature. Despite that fact, it is important to be aware that the risk of nosocomial transmission of *M. tuberculosis* depends on local prevalence of TB. Thus, such an assumption can be made that smaller hospitals will probably have lower rates of LTB prevalence when compared to bigger hospitals with infectious diseases departments. Further studies are necessary to establish this connection.

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PERITONITE FECAL SECUNDÁRIA A PERFURAÇÃO IATROGÉNICA DO CÓLON

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Introdução: A peritonite fecal resulta de um processo infeccioso secundário à presença de fezes na cavidade peritoneal. Reportamos um caso de peritonite fecal secundária a perfuração cólica, não detetada durante cirurgia urológica.

Caso clínico: Doente de 17 anos, 4º dia pós-operatório de correção de varicocele, sob piperacilina/tazobactam e metronidazol, transferido para o CHUSJ por quadro de febre, dor abdominal e enfisema subcutâneo escrotal, elevação dos parâmetros inflamatórios e evidência imagiológica de ascite abdominal e pneumoperitoneu.

À admissão apresentava sinais de choque séptico, tendo sido associada vancomicina ao esquema terapêutico e realizada laparotomia exploradora; constatada peritonite fecal secundária a perfuração iatrogénica do cólon descendente, realizada rafia da laceração e colostomia transversa. Por isolamento de *Escherichia coli* e *Candida albicans* no líquido peritoneal, associou-se anfotericina B.

Em D5, por dor e sinais inflamatórios abdominais, repetiu TC que revelou abscesso subhepático. Foi submetido a laparotomia para lavagem peritoneal, e no líquido isolado novamente *C. albicans*.

Por manter febre sem identificação de outros focos, realizada cultura peritoneal em anaerobiose e alterado esquema para meropenem e fluconazol; isolado bacilo Gram-negativo, identificado pelo método de MALDI-TOF MS como *Parabacteroides distasonis*. Em D18 foi transferido dos cuidados intensivos pediátricos para outra instituição; teve alta em D40 com apirexia sustentada.

Discussão: A peritonite secundária à contaminação por conteúdo gastrointestinal é geralmente polimicrobiana, com predomínio de Enterobacterales na fase aguda e anaeróbios e fungos na tardia.

Trata-se do primeiro isolamento peritoneal descrito de *Parabacteroides distasonis*, um bacilo Gram-negativo anaeróbio obrigatório, comensal do intestino humano, associado a taxas de resistência antimicrobiana superiores a outros anaeróbios intestinais.

O caso expõe as dificuldades diagnósticas da peritonite e suas implicações clínicas. Salienta-se a importância do envio do líquido peritoneal em condições de anaerobiose e do papel do MALDI-TOF na identificação de anaeróbios, dado que alguns grupos são fenotipicamente similares e frequentemente mal identificados por metodologias clássicas. O atraso diagnóstico contribui para antibioterapia prolongada e propicia evolução da infeção peritoneal, com favorecimento de agentes resistentes e peritonite terciária, agravando o prognóstico e risco de mortalidade.

10-YEAR STUDY OF LISTERIA MONOCYTOGENES IN A TERTIARY HOSPITALNídia Neves¹; Yaqueline Freitas¹; Cheila Plácido¹; João Freitas¹; Mariana Fardilha¹; Miriam Sousa¹; Nuno Canhoto¹¹Hospital Central do Funchal, SESARAM E.P.E.

Background: Listeriosis is an infection caused by the bacteria *Listeria monocytogenes* (Lm), a non spore forming gram positive bacilli, commonly associated with the consumption of contaminated food. Although uncommon, infections can be severe, such as sepsis, meningitis or encephalitis, especially in immuno compromised patients and newborn, and may reach a 30% mortality rate.

The antibiotic of choice is ampicilin or penicillin. Gentamicin can be used for synergy. Alternatively, treatment with trimethoprim sulfamethoxazole is appropriate.

In Portugal, listeriosis has been a notifiable disease since 2014 through the National Epidemiological Surveillance System.

Our aim is to know the reality of a tertiary hospital regarding Lm infections in the last decade and to verify if the antibiotic de escalation was effected after the identification of the agent.

Materials/methods: Statistical survey of Lm isolations in samples sent to our laboratory between October 2009 and October 2019 using Modulab[®] v2.3.08 and Atrium[®] v22.0.5.0 softwares. Data were analyzed using Microsoft Excel 2013[®] software.

Results: During the studied period, Lm was isolated from 23 patients. The mean age was 65.3 years (minimum of 42 and maximum of 91). The studied population consisted mainly of women (n=14, 61%). 10 patients (43%) were immuno compromised. Death was verified in 9 cases (39%). Antibiotic de-escalation occurred in 15 cases (65%) but not in 2 cases (9%); in the remaining 6 cases (26%) it was not possible to evaluate due to patient death before the agent identification. In 17 patients the microorganism was isolated from 1 sample and 6 patients had isolates from 2 different samples, with a total of 29 isolates. Microorganisms were isolated from blood cultures (n=16, 55%), cerebrospinal fluid (n=8, 28%), ascitic fluid (n=4, 14%) and pleural fluid (n=1, 3%).

Conclusions: During the last decade, the number of listeriosis in our hospital has been low, but the mortality rate was very high, so it is essential to know how to recognize, diagnose and treat this infection, especially in immuno compromised patients, elderly, pregnant and newborn infants. The rate of de escalation was high, demonstrating awareness among physicians about antibiotic stewardship, which is extremely important concerning the control of antimicrobial resistance.

RISK FACTORS FOR MRSA RESPIRATORY INFECTION IN INPATIENTS OF A TERTIARY HOSPITAL: ENVISAGING SCREENING FOR RISK GROUPS AT ADMISSIONÂngela Maresch¹; Ricardo Ribeiro¹; Micaela Baptista²; João Frade¹; Cristiana Canha¹; Catarina Chaves¹; Henrique Oliveira¹; João Pego¹; Fernando Rodrigues¹¹Centro Hospitalar e Universitário de Coimbra²Instituto Português de Oncologia

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) to hospital wards enhances infection control and reduces antimicrobial empirical treatment. *S. aureus* is a frequent cause of respiratory infection requiring hospital stay, imposing significant diagnostic and therapeutic challenges.

We aimed to identify risk factors for MRSA colonization/infection amongst adult inpatients identified with *Staphylococcus aureus* in respiratory samples during the last year.

Empirical analysis was used to assess the association between patient's demographic information, ward and length of hospital stay, and MRSA. MALDI-TOF was used for the identification, followed by VITEK antibiotic susceptibility testing to identify resistance to oxacillin.

After exclusion of duplicates, 467 patients were included (median 77.0, IQR 63.0-84.0 years of age, male 61.7%). 54.8% of *S. aureus* were MRSA. Univariate logistic regression revealed a dose-effect association for increasing age, time duration from admission until *S. aureus* identification, medical or surgical ward (all with P-trend <0.0001).

Nonetheless, in the multivariate analysis, length of stay lost significance (p=0.34).

Overall, the main factors predicting increased risk for MRSA were increasing age and staying in a medical ward.

We concluded that age >84 YO and being in a medical ward helped predict MRSA in adult inpatients at our hospital. Gender and length of stay were not as strong predictors, suggesting preponderance of patient related factors. These findings could be considered to guide MRSA screening in adult inpatients with suspected respiratory infection.

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CLINICAL, EPIDEMIOLOGICAL AND MICROBIOLOGICAL FEATURES OF STREPTOCOCCUS PYOGENES INVASIVE INFECTION - 10 YEAR RETROSPECTIVE REVIEW

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Background: *Streptococcus pyogenes* (Group A *Streptococcus*, GAS) is a Gram positive cocci, human restricted bacterium, responsible for uncomplicated infections. However, it may cause more severe, life-threatening invasive illnesses, such as necrotizing fasciitis and pneumonia. Invasive infections (iGAS) are a severe threat for predisposed patients, associated with significant mortality. We aim to describe the epidemiology, clinical and microbiological features of iGAS in adult patients.

Methods: A retrospective cohort study in adult patients with iGAS at a tertiary university hospital in the last 10-year period. iGAS was defined as microorganisms isolated from a sterile site. Data was collected from medical records. Comparisons between groups (survivor Vs non-survivor group) were made regarding demographics, comorbidities and clinical data.

Results: A total of 58 patients were included (51,7% women) with mean age of 66,1±17,74 years. The skin was the most common primary site of infection 46,6% cases and the lungs in 34,5% patients. 13,8% had bacteremia of unknown origin and 5,2% primary ear infection. Cardiovascular risk factors were the most frequent comorbid conditions (67,2%, hypertension; 50% dyslipidemia and 34,5 % type 2 diabetes mellitus). More than half of the patients required intensive care and a third mechanical ventilation. Regarding antibiotic therapy, 3^o generation cephalosporins were mainly used, and were found resistance in 8 cases to quinolones and 5 to penicillin. In-hospital mortality rate was 32,8. Alcohol abuse, cirrhosis and previous stroke were significantly more frequent in non-survivors' group, and also, intensive care admission and the need for organ support.

Conclusions: Although uncommon, iGAS is a severe condition with high mortality rate and the need of intensive care, demonstrated in our study in a significant proportion of patients. The most common presentations and comorbid conditions were similar to those reported in other studies. Certain risk factors for invasive infections such as age' extremes, immunosuppression, diabetes mellitus and loss of skin integrity have been previously established. In our cohort, alcohol abuse, cirrhosis and previous stroke seems to be factors related to poor outcome.

EVOLUTION OF THE FLU TREND IN THE 2019-2020 SEASON AT COIMBRA HOSPITAL AND UNIVERSITY CENTER

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Introduction: Annual influenza epidemics are a major cause of morbidity and mortality, especially in the most susceptible groups. Seasonal flu is estimated to affect about 1 to 15% of the world's population. The laboratory diagnosis of influenza is a fundamental instrument for public health, both in the individual approach and in epidemiological surveillance.

Objective: To determine the trend of influenza in the 2019-2020 season in a tertiary hospital.

Methods: Retrospective study, from 1 November 2019 to 26 February 2020, in which all requests for influenza research were included. Samples were collected by oro/nasopharyngeal swab and processed on the GeneXpert® (Cepheid) equipment, using the Xpert® Flu/RSV Assay. Positive samples were characterized according to age, type of virus (Influenza A, Influenza B or Respiratory Syncytial Virus - RSV) and weekly distribution.

Results: Between 2019-2020, from 1804 requests for suspected flu, only 30.9% of the tests were positive for viral respiratory infections tested by this real-time multiplex PCR assay. Of these, 59.7% were identified as Influenza A virus (n=333) and 16.1% as Influenza B virus (n=90). The peak incidence of Influenza B occurred in the third week of December 2019, while the peak of Influenza A occurred in the first week of February 2020. 149 patients with Influenza A were over 65 years old and 10 were under 18 years old. RSV was detected in 144 patients, with a peak incidence in the fourth week of January. The median age of patients with Influenza A, Influenza B and RSV was: 62 years [min 2 months-max 97], 33 years [min 1-max 92] and 79 years [min 3 weeks-max 102], respectively. Nine concomitant infections with these viruses were identified, mainly Influenza A and RSV (n=6). The highest percentage of orders (46.1%) and the highest number of positive tests overall was carried out in January 2020 (n=303).

Discussion: The diagnostic test for influenza and RSV infection based on the amplification of nucleic acids is fast, accurate and easy to perform. The Influenza A virus was the most frequently detected, showing a gradual increase from the beginning of January to the first week of February. It was observed a decreasing trend until the end of February.

EPIDEMIOLOGICAL CHARACTERIZATION OF CARBAPENEMS RESISTANT ENTEROBACTERIALES IN OUR INSTITUTION

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Introduction: The spread of carbapenem-resistant microorganisms has been classified as a public health problem by the World Health Organization (WHO). Rapid and assertive detection of resistance mechanisms is crucial to allow for the implementation of adequate therapy and infection control measures. Microbiology laboratory plays a very important role in this process.

Objectives: Epidemiological characterization of carbapenem-resistant Enterobacterales (CRE) isolated in clinical samples in our institution, in 2019, whose coverage area is 141626 inhabitants.

Material and methods: The identification and antibiogram were performed by Vitek 2 compact® system (bioMérieux) and the minimum inhibitory concentration of meropenem and ertapenem was confirmed through strips with a gradient of concentration Etest® (Werfen), applying EUCAST-2019. The phenotypic detection of carbapenemases production was detected by Rapidec® CarbaNP technique (bioMérieux) and genotypic determination by real-time PCR was obtained for the most frequent genes (KPC, VIM, IMP, NDM and OXA-48) with the system a Xpert® Carba-R (Cepheid).

Results: 42 CRE strains were isolated from in-patients or patients with recent hospitalization. Clinical and demographic data are described in table 1 and the phenotypic and molecular characterization in table 2.

Conclusions: Most frequent CRE identified was *Klebsiella pneumoniae*. Production of carbapenemases was confirmed genotypically in 97.6% of the CRE, being mostly KPC. All isolates had resistance to ertapenem, while resistance to meropenem was only detected in 21.4% of the isolates. Three *Klebsiella pneumoniae* OXA-48 isolates were not detected by Rapidec® CarbaNP technique. Taking into account the methods employed, it was not possible to conclude about the resistance mechanism in the *Escherichia coli* isolate.

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URINARY TRACT INFECTIONS BROUGHT ON BY STREPTOCOCCUS GALLOLYTICUS

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Introduction: Urinary Tract Infections (UTI), one of the most common bacterial infections, is more prevalent in women. The etiology of UTIs is well established, with *Escherichia coli* being the predominant pathogen. Some noteworthy microorganisms found in UTIs are *Streptococcus gallolyticus*, *Streptococcus bovis* group.

Description: The Clinical Microbiology Laboratory looked to study the number of *Streptococcus gallolyticus* isolates in urine cultures from January 2018 to May 2019. A total of 9 patients' medical histories were analyzed, 8 female and 1 male, aged between 43 and 89. Six were over 80 years old, all female. All cases were identified using MALDI-TOF and Antimicrobial Susceptibility Tests (AST) were performed according to EUCAST. Since the beginning of 2019, 4 *Streptococcus gallolyticus* cases were identified. Three cases were females between the ages of 83-89. All females shared a similar medical history, had been previously diagnosed with hypertension, type 2 diabetes and frequent UTI. The fourth female, a 53-year-old, suffered from frequent UTI and was diagnosed with psychiatric disorders. The AST results in all patients revealed sensibility to Penicillin, Ceftriaxone, Cefotaxime and Vancomycin and only 2 patients were resistant to Clindamycin.

Clinical Case: An 87-year-old woman goes to Accident and Emergency complaining of nausea, frontal headache and abdominal pain. Patient has a history of Hypertension, Type 2 Diabetes and Dyslipidemia. On physical examination, she was afebrile and hypertensive (TA:180/80 mmHg). Complementary diagnostic Tests showed normal blood count, liver function and C-Reactive Protein 1,1; Thoracic X-ray and Cerebral CT scan without significant changes. Type II Urine Test came back positive for leukocyturia (500 leukocytes/HPF). Lastly, a urine culture was requested, the patient was discharged and prescribed Phosphomycin. The bacteriological examination of the patient's urine revealed a colony growth greater than 10⁵ CFU / ml suggesting a UTI. The Antibiogram result was Sensitive (S) to Ceftriaxone, Penicillin, Cefotaxime, Vancomycin and Resistant (R) to Clindamycin.

Conclusion: *Streptococcus gallolyticus*, a rare entity, appears to be emerging as a potential UTI agent, and further study of this pathogen is warranted in future populations.

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A CASE OF SOFT TISSUE INFECTION BY PASTEURELLA CANIS

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Introduction: *Pasteurella canis* belongs to the oropharyngeal microbial flora of domestic animals, particularly dogs. They are characterized by being small, nonmotile, glucose fermenters, Gram negative bacilli from the Pasteurellaceae family. They are potentially pathogenic in humans, causing mainly soft tissue infections following a pet bite.

Clinical Case: A male child of 10 years old, without relevant medical record, seeks medical attention at the emergency department for an abscessed inflammatory process at the proximal region of the 1st finger of his right hand (thenar region). He presented with local pain, heat, redness and edema. He refers a 2 days old dog bite on the affected area. Analytically, an increase of inflammatory parameters was observed (Reactive Protein C: 1,41mg/dl; Leukocytes: 10,30x10³/uL), without other relevant analytical changes. He is hospitalized for surgical drainage of the abscess, collecting pus for bacteriological examination. Cultural growth was observed, in chocolate agar media, of convex, smooth and nonhemolytic colonies of Gram negative bacteria. The strain was identified as *Pasteurella canis* by mass spectrometry technique - MALDI-TOF. He started treatment with amoxicillin/clavulanic acid in combination with clindamycin, with good response and clinical evolution.

Conclusion: This work intends to highlight the contribution of the microbiology laboratory in order to establish the proper diagnosis and appropriate treatment of the patient with infection by animal bite.

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EPIDEMIOLOGY OF GRAM-NEGATIVE BACILLI ISOLATED FROM POSITIVE BLOOD CULTURES FROM PATIENTS ADMITTED AT A TERTIARY CARE HOSPITAL IN PORTUGAL.

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Introduction: The emergence and rapid spread of antibiotic resistance in pathogens represent a threat to health systems. Gram-negative bacilli have the ability to produce enzymes that inactivate clinically relevant antibiotics such as β -lactams. Extended-spectrum β -lactamases (ESBLs) and Carbapenemases are the most important enzymes.

Objective: The aim of this study was to evaluate the antimicrobial susceptibility pattern of Gram-negative bacilli isolated from positive blood cultures and to perform the molecular and phenotypic characterization of β -Lactamases enzymes. This study was carried out at Centro Hospitalar Universitário de São João (CHUSJ), Portugal.

Methods: Enterobacterales family and non-fermenting Gram negative bacilli (N-FGNB) isolates during October and November of 2019 were identified by Vitek[®] MS System (bioMérieux) and antimicrobial susceptibility testing was performed using Vitek[®] 2 (bioMérieux). Only the first isolate per patient from were consider. Detection of most commonly Carbapenemases genes (*bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48 e *bla*IMP-1) was performed using Xpert Carba-R Assay, GeneXpert (Cepheid[®]) and Vitek[®] 2 System was used to identify ESBL producers.

Results: During a period between October and November from 2019, we have isolated 181 Gram-negative bacilli isolated, 144 (79.5%) were from the Enterobacterales family, 31 (17.1%) were N-FGNB and 6 (3.3%) other Gram-negative bacilli. The most frequently isolated species were *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa*. Regarding the hospital department, 30.2% of isolates were from the outpatient department, 24% from medical and surgical wards and 14.85% from Intensive Care Units. About the susceptibility profile, 69.1% presented a resistance pattern at least for one antimicrobial drug group (84.7% in Enterobacterales and 15.3% in N-FGNB) Four strains (2.2%) produced carbapenemase: *P. aeruginosa* VIM, *K.pneumoniae* OXA-48 and two isolates *K.pneumoniae* KPC. ESBL production was observed in 25 isolates (17.3%) from Enterobacterales family.

Discussion: This study demonstrates that Enterobacterales is the most frequently isolated family, with the highest prevalence of antimicrobial multiresistance in patients with bacteraemia admitted in Intensive Care Units.

RECURRENT SALMONELLOSIS: CASE REPORT

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Introduction: Salmonella spp. is an Enterobacteriaceae, which is responsible for Salmonellosis. Serotyping complements the identification, it's based on the detection of somatic and flagellar antigens. Salmonellosis may present with large spectrum of clinical infection from gastroenteritis to enteric fever.

Severe infections can invade into the blood stream and cause bacteremia with the possibility of disseminated focal infection. Nontyphi Salmonella bacteremia is uncommon, severity and risk of translocation are often determinate by the virulence and host immunity, mainly in elderly and immunosuppressed patients.

Salmonella typhimimum is the most common in such circumstances. Our aim is to present a case of recurrent bacteremia by Nontyphi Salmonella.

Case Report: Female, 87-years-old. History of hospitalization, 1 month ago, for urinary tract infection by Salmonella enterica typhimurium O:4, which apparently improved under Piperacillin-Tazobactam antibiotics. She went to the emergency room (ER) for prostration and fever.

Analytically, she presented normocytic normochromic anemia (hemoglobin 9.6g/L), thrombocytopenia (43x10⁹/ml) with leukopenia (3,4x10⁹/ml) and an increased C-reactive protein; hyperglycemia, urea 87mg/dl, creatine 2.14mg/dl, hyperkalemia (5.6mEq/l); liver function: γ glutamyl-transferase 554U/l and alkaline phosphatase 251U/l. Past medical history: idiopathic chronic liver disease; diabetes mellitus type 2; chronic kidney disease; hypertension; interstitial lung disease.

She was admitted at the Medicine Unit with the diagnosis of pneumonia of the right lower lobe and increased chronic kidney disease with hyperkalemia. Blood cultures was collected in the ER and empirically starts Trimethoprim and Sulfamethoxazole.

Salmonella enterica typhimurium was isolated on blood agar from blood culture, was serotyped in a reference laboratory, as group B with antigens O:4,H:i. According to the antimicrobial sensitivity test, she started Cefotaxime with the dose adjusted to renal function, with clinical improvement.

Discussion: Some conditions predisposing to Salmonellosis include infection with human immunodeficiency virus, diabetes mellitus, prolonged corticosteroid therapy, alcohol abuse, chemotherapy and some malignancies. The resistance of nontyphoid Salmonella to antibiotics previously considered effective, such as Ampicillin, Chloramphenicol, Sulfamethoxazole-Trimethoprim, has increased. Experience has shown the effectiveness of third generation cephalosporins and quinolones.

QUALITY OF SPUTUM SAMPLES FOR THE DIAGNOSIS OF LOWER RESPIRATORY TRACT INFECTIONS

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Introduction: Laboratory diagnosis of lower respiratory tract infections (LRTIs) is mainly performed through cultivation of sputum samples. However, microbiological diagnosis of these samples is complicated due to the difficulty of obtaining a sample that is not contaminated with the usual oropharyngeal microbiota. It is of paramount importance that the clinical laboratory ensures the quality of the processed samples.

Objective: This study was done to evaluate the association between the cytological examination and the cultural examination for the diagnosis of LRTIs, at our institution, in 2019.

Material and methods: 1707 sputum samples were subjected to a Leishman staining cytological assessment, seen under the low power field magnification (LPF), considering the presence of squamous epithelial cells (SECs) and polymorphonuclear cells (PMNs), according to the criteria of Murray & Washington. Samples with more than 25 SECs/LPF were not sown.

Microbiological characteristics, clinical and treatment data were evaluated.

Results: 46% (786) of the samples were automatically excluded for cultural examination due to the presence of more than 25 SECs/LPF. 40.8% (696) were samples with <10 SECs/LPF, of which 10.5% (73) were considered positive and of these only 34,2% (25) had > 25 PMNs/LPF. 13.2% (225) were samples with 10-25 SECs/LPF, of which 5.3% (12) were considered positive, and of these only 8.3% (1) had > 25 PMNs/LPF. In both groups (<10 SECs/LPF and 10-25 SECs/LPF), in samples with more than 25 PMNs/LPF, about 92% and 100% fulfilled criteria for infection. The group of samples with <10 SECs/LPF and group 10-25 SECs/LPF had 67.2% (468) and 73.8% (166), respectively, of mixed microbiota, regardless of the number of PMNs.

Conclusion: A similar high percentage (67.2% and 73.8%) of mixed flora, suggestive of oropharyngeal contamination, was found in both groups (<10 SECs/LPF and 10-25 SECs/LPF). For samples considered positive, we are faced with a problem of differentiation between LRTI and colonization of the upper respiratory tract with potentially pathogenic microbiota, which appears mainly in hospitalized patients and those treated with antibiotics. The results obtained suggest that the presence of PMN is an indicator of infection.

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PARASITIC MORPHOLOGY: IMPROVEMENT OF LABORATORY PERFORMANCE BY PARTICIPATING IN AEQ?

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Introduction: Parasitic diseases are responsible for morbidity and mortality worldwide, according to the World Health Organization. The participation of clinical laboratories in External Quality Assessment programs allows obtaining reliable results in parasite identification, assisting clinicians on correct diagnosis.

Objective: Assess the improvement of PNAEQ participants in microscopic identification of parasites scheme during eight years (2011 to 2018).

Methodology: Statistical analysis was performed using Excel Office 365, using test. Participants results of PNAEQ schemes, were organized by sample type (faecal or blood), laboratory characterization (outpatient or hospital) and year.

Results: For faecal samples the performance varied between 21% (>1 parasite) - 90% (1 parasite) and 23% (>1 parasite) - 88% (1 parasite) for outpatient and hospital laboratories, respectively. However, participants identified correctly pathogenic parasites, even when other commensal parasite were present. In general, the performance for samples containing protozoan was better than the ones with helminths as expected. For blood samples, the performance was similar, varying between 38-80% and 50-78%, for outpatient and hospital laboratories, respectively.

Conclusion: According to the results, hospital and outpatient laboratories performance was similar for both type of samples. The performance for samples with more than one parasite was lower regarding the great difficulty to identify correctly parasites. In order to improve the performance of the participants and contribute to a correct diagnosis PNAEQ will continue to be involved in continuous education by providing theoretical-practical courses and reports with scientific information.

Keywords: laboratory, ambulatory, hospital, performance, parasite

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BACTERIA IN THE PERIPHERAL BLOOD FILM – ONE MORE STEP TO SEPTICEMIA IDENTIFICATION

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Introduction: A rapid diagnosis is essential in the management of septicemia. The diagnosis is confirmed when a microorganism is identified in the blood. The blood culture plays an important role in its diagnosis, but the result could take some time. The peripheral blood film (PBF) has been sometimes reported as a simple method that can be used to hasten the confirmation of septicemia, enabling clinicians to select a more specific treatment. However, the detection of bacteria in the PBF may in some cases occur only in overwhelming septicemia.

The objectives of this work are to identify the most frequent bacteria identified in a PBF and guidelines that can help us when a PBF is performed. This information is usually related with case reports description.

Materials and Methods: Using scientific databases, a search was performed to find scientific articles describing the identification of bacteria in the PBF and the cautions that we must bear in mind when validating these findings.

Results: The bacteria identified were in 47% of cases Gram positive cocci, 41% Gram negative bacilli, 6% Gram positive bacilli, 6% Gram negative cocci.

During PBF examination, the typical Gram reactions, morphology and arrangements of the observed bacteria may give the presumptive identification.

The major criteria for the diagnosis of septicemia based on a PBF are the presence of bacteria inside leucocytes. If only extracellular microorganisms is observed, they have to be interpreted carefully because those could be contamination in vitro such as artifacts or result from contamination during sample collection. Other findings that can help us to identify a infection are the presence of toxic granules, cytoplasmic vacuoles, and Döhle inclusion bodies, these alterations in neutrophils could be used to distinguish true bacteremia from specimen contamination.

Conclusions: PBF is much more insensitive when compared with blood cultures, but is a simple, rapid and inexpensive method as a first step to bacteria identification, enabling clinicians to select a more specific treatment.

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CAMPYLOBACTER SPP IN CENTRO HOSPITALAR DE TRÁS-OS-MONTES E ALTO DOURO: AN OVERVIEW

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It is known *Campylobacter* spp is the cause of most bacterial enteritis. In our hospital, it is an agent frequently identified. The aim of this retrospective observational study is to characterize the epidemiology, clinical manifestations and treatment considering the in vitro susceptibility profile of this agent in our population. To achieve these objectives, we analyzed all the clinical cases identified in 2018.

A total of 71 cases were identified, most in stool samples and almost all of them from the paediatrics emergency department. However, 7% of patients presented with bacteraemia, all adults, and half of them with chronic liver disease. *C. Jejuni* was the specie most commonly identified, predominantly in the months of May, followed by June and July. Fever with bloody diarrhoea was the most frequent clinical presentation, but in 63% of cases no antimicrobial therapy was needed, as the infection was self-limited. Regarding antimicrobial susceptibility, we found that *Campylobacter* spp isolated in our hospital was resistant to tetracyclines in 85% of cases, also to quinolones in 94% of cases and to ampicillin in 80% of cases. Despite this, all the isolated microorganisms maintained susceptibility to erythromycin, amoxicillin with clavulanic acid, gentamicin and carbapenems.

Campylobacter spp is a frequent cause of infection and despite being self-limited in most cases, some patients still need antimicrobial therapy. This study reflects the urge to a proper use of this type of drugs, as we are confronted with a high rate of resistance to antimicrobials.

INFLUENZA VIRUS: LABORATORY CONFIRMATION OF INFECTION AND EPIDEMIOLOGICAL FINDINGSCátia Fortes¹; Andrea Arias Reboredo¹; Elmano Ramalheira¹¹Centro Hospitalar do Baixo Vouga

Background: Influenza A and B viruses have a worldwide distribution and cause seasonal epidemics of disease in humans every year. Illness ranges in severity sometimes requiring hospitalization and even leading to death. Laboratory confirmation of influenza virus infection is commonly performed using molecular assays and antigen detection tests.

The aim of this retrospective study was to evaluate the request profile of laboratory confirmation tests of influenza, since the introduction of molecular assays, in our hospital, in 2016. Additionally, we assessed some epidemiological tendencies.

Materials/Methods: The present study was carried out between January 2016 and December 2019 covering three influenza seasons. Two rapid immunochromatographic assays were used for detection of influenza virus antigens: SD BIOLINE Influenza Antigen and SD BIOLINE Influenza Ag A/B/A(H1N1) Pandemic (Standard Diagnostics). Seegene AllplexTM Respiratory Panel 1 was used for molecular biology assays.

Results/Discussion: During the timeframe of this study the number of requests for rapid influenza diagnostic test was significantly higher compared to the PCR based test (7182 and 107, respectively). For rapid test most samples were collected from individuals of pediatric age (n=5037) and from outpatients (n=6857). The immunochromatographic test revealed to be the clinicians' first choice, as expected. Nonetheless, molecular biology is a more specific and sensitive tool when compared to the former test, which allows confirming the infection when the rapid test is negative and there is still a high level of suspicion. Additionally, it allows the identification of influenza subtypes not included in the rapid assay. Globally, influenza A was the most common type identified. Influenza A(H1N1) pandemic strain was detected in 21 cases, however the number of positives has been decreasing. Isolated infection by A type influenza was the most prevalent one in epidemics season. Influenza infections outside the normal influenza epidemics were most frequently caused by influenza B viruses' type. Although co-infection was rare, cases of influenza A and B co-infection were found, even outside the influenza seasons. Within molecular tests performed, influenza A was the most prevalent being H3 subtype the most common.

ANTIMICROBIAL SENSITIVITY ANALYSIS OF CARBAPENEMS RESISTANT ENTEROBACTEREALES IN OUR INSTITUTIONFátima Vale¹, Rita Gralha¹, Cátia Oliveira¹, Joana Ramos¹, Ana Santos¹, Adriana Rabaça¹, Filipa Borges¹, Cláudio Roque¹¹ULS Guarda

Introduction: Recently the isolation of Enterobacterales with reduced carbapenem-resistant Enterobacterales (CRE) has grown significantly. This resistance is a serious problem for public health and can be caused by different mechanisms, such as the production of carbapenemases or the presence of mixed mechanisms of resistance to antibiotics carbapenems (carbapenemases, ESBL and / or AmpC plus loss of porins). The importance of healthy carriers in the persistence and dissemination of the CRE and the difficulties in detecting some of these strains represents a great challenge for the clinical laboratory of microbiology.

Objectives: The objective of this study is to analyze the phenotypic susceptibility profile of strains, in different periods of time, taking into account the antimicrobial administered.

Material and methods: Were selected three patients with CRE strains, namely *K. pneumoniae*, and the antimicrobial profile was evaluated. The identification and antibiogram of the strains was performed by VITEK 2 Compact[®] system (bioMérieux) and the determination of the minimum inhibitory concentration (MIC) of the carbapenems (ertapenem and meropenem) by Etest[®] gradient diffusion strips (Werfen), according to the cut-off points of EUCAST-2019.

Results: During the period under study, phenotypic susceptibility profiles of strains isolated from hospitalized patients or patients with recent hospitalization were evaluated. The phenotypic and molecular characterization of the isolates is presented in Table 1. The files referring to each patient were consulted to check the antibiotics applied.

Conclusions: In case A we have had an increase in resistance. In July, the *K. pneumoniae* strain was susceptible to 2nd, 3rd, 4th generation cephalosporins and carbapenems. In September 2019 the patient had a strain with KPC enzyme. The patient has undergone treatment in July and August with amoxicillin/clavulanic acid, cefuroxime, levofloxacin, co-trimoxazole, which presupposes antibiotic pressure. In case B there was an increase in MIC compared to carbapenems. In case C, the patient had an OXA-48 strain, was treated with cefuroxime in September 2019 and in October it was no longer detected. With the exception of case C, there was an increase in resistance in different classes of antibiotics and a decrease in therapeutic alternatives, most likely due to the selective pressure exerted by the use of antibiotics or by acquisition of resistance plasmids.

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CAT BITE: A CASE REPORT OF SOFT TISSUE INFECTION BY PASTEURELLA SPP

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Pasteurella spp are usually encountered after cat or dog bites. It is a Gram negative bacilli that does not grow on the MacConkey agar. An unusual feature of this microorganism is that it has no intrinsic resistance to penicillin, contrary to other clinically relevant Gram negative bacilli.

We report the case of a healthy young woman, who was bitten by a cat and developed an infected wound on the hand. The infection spread throughout the arm, and she was admitted for treatment with intravenous antibiotics. Pus was collected from the bite and sent to our laboratory. On the Gram stain, Gram negative bacilli were observed, but nothing grew on the MacConkey agar. From the blood agar we were able to isolate catalase and oxidase positive colonies, then an identification of *Pasteurella dagmatis* was made.

Given its susceptibility to penicillin, the empiric antimicrobials used, which included Piperacillin with Tazobactan, were sufficient to treat the patient.

This is a rare case in our laboratory, and it reflects the importance of a proper microbiologic diagnosis, as we can straighten the antimicrobial spectrum and still effectively treat the patient.

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BARTONELLOSIS: A SUGGESTIVE CASE REPORT IN AN ADOLESCENT PRESENTING WITH CERVICAL TUMEFACION

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Cat scratch disease is a subacute bacterial infection of which *Bartonella henselae*, a gram-negative rod, is the etiological agente. The possible vectors are arthropods, such as flea, and the main reservoir of the bacterium is the young cat. It is transmitted by the scratch or bite of infected cats. Although pathogenesis remains unclear, this disease is characterized by regional lymphadenopathy, proximal to the site of inoculation, being one of the most common causes of lymphadenopathy with subacute or chronic evolution in children and adolescents. Here, is described the case of a teenage girl with a left submandibular tumefaction with 8 months of evolution after upper respiratory infection. There were no associated symptoms and no contact with sick people. She refered frequent contact with cats. Serologic testing was compatible with past infection by *Bartonella henselae*. The diagnosis of this disease is based on clinical suspicion and laboratory confirmation by serology (antibodies to *Bartonella henselae*). The differential diagnosis should take into account other infectious and non-infectious causes of isolated lymphadenopathy and, in this context, ganglion biopsy with subsequent histopathological examination and PCR (polymerase chain reaction) analysis may be useful. Treatment is controversial given the benign and self-limited course of the disease, in the vast majority of cases. However, in case of atypical manifestations and/or is present systemic involvement, antibiotherapy may be recommended.

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CANDIDA SPP ISOLATED FROM URINE

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Introduction: Candida spp. urinary tract infections are an increasingly prevalent nosocomial problem with uncertain significance, once it represents a challenge as to whether Candida spp. isolation represents colonization or, lower or upper urinary tract infection. Confirmation of positive Candida spp urine culture can be confirmed by a second urine sample to rule out colonization or contamination.

C. albicans, C. glabrata and C. tropicalis are the most often Candida species isolated from urine samples.

The most important risk factors for severe fungal infection are frequent or long-term medical care exposure or treatments with antibiotics. Antibiotics can contribute to colonization by Candida spp. by suppressing endogenous bacterial microbiota.

Objective: To assess what are the most frequent species of Candida isolated from urine samples.

Materials and Methods: We analysed a total of 312 results of urine cultures (of the year 2019) where Candida spp. was isolated to assess which were the most frequently isolated species.

To assess the identification of Candida spp. with the age of the patients.

Results: Candida spp. was isolated in 67.7% of patients over 70-year-old. The number of patients under 50-year-old only accounted for 9.5%.

By gender, 55.8% of the isolates were from women and 44.2% from men.

Candida albicans (64.4%) was the most often isolated Candida spp. in urine followed by C. glabrata (19.9%) and C. tropicalis (6.1%). Other isolates included C. parapsilosis, C. krusei, C. lusitaniae, C. kefyr.

C. albicans, C. glabrata and C. tropicalis were isolated, respectively, in 81.6%, 95.1% and 94.7% of patients over 70-year-old.

Conclusion: We found that C. albicans, C. glabrata and C. tropicalis were the most frequent Candida spp. isolated from urine samples in our Hospital Center. In addition, our study showed that the isolation of Candida spp. is more frequent in patients over 70-year-old. These results are similar to other studies.

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HBA2': A S-WINDOW SMALL PEAK

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Introduction: Haemoglobin A2 prime (HbA2') is the commonest delta chain variant of HbA2, occurring in a small percentage of individuals of African descent. High performance liquid chromatography (HPLC) identifies it as a small S-window peak. Although haematologically and clinically silent, it needs to be identified in certain contexts, especially during the workup of beta thalassemia (BT).

Clinical Case: A 17 months-old African female is referred to consultation for anaemia workup. During the follow-up, a HPLC analysis is made, showing normal results except for a HbA2 of 1.3% and a 1.2% peak on the S-window. The sample tube didn't follow a tube containing any detectable HbS, therefore a carryover was excluded. The patient was never transfused. The S-window peak wasn't reported, and reevaluation was recommended. Six months later, the same 1.2% S-window peak was detected. Again, the tube that preceded the sample tube didn't contain any detectable HbS, and after it was repeated, results were identical. This time, the S-window peak was reported as a probable delta chain variant of unlikely clinical significance.

Discussion: Due to HbA2' eluting in the S-window, it is likely that any small discreet peak in that position is A2'. Clinical pathologists should sum the two peaks (A2 + A2') to have an approximate idea of what the total A2 is. If believed that the total A2 falls in the normal range, it should be reported as a delta chain variant of unlikely clinical significance. If the total A2 appears high, BT should be confirmed by gene sequencing. This assumes the patient has not had transfusion, as it is possible that small fractions of variant haemoglobins occur due to transfused blood, and that it is not a highly exchange transfused sickle cell disease patient who can have very low levels of HbS. Oddly shaped peaks are also unlikely to be A2' which has a discrete peak shape like HbA2. The absence of HbA2 and presence of a relatively small S-window peak, should also remind us of a possible homozygous HbA2' state.

Conclusion: Although easily overlooked and dismissed by clinical pathologists, one should always be careful when evaluating small discreet peaks in the S-window, to avoid failing to diagnose BT.

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ADULT HEMOPHAGOCYtic SYNDROME SECONDARY TO DRESS SYNDROME: A CASE REPORT

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Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a rare, severe hyperinflammatory hiperferritinemic syndrome due to uncontrolled macrophage and cytotoxic T cell activation. In adult patients, it mainly occurs secondary to malignancies, infections or autoimmune disorders. We present a case of a HLH syndrome secondary to DRESS syndrome.

Case Description: A 56-year-old man with history of smoking and peripheral artery disease, medicated with acetylsalicylic acid 100mg presented with pain, redness and swelling of the left hallux finger, therefore was submitted to amputation of the respective finger and initiated antibiotic therapy with piperacillin-tazobactam and vancomycin. The patient presented with skin rash, fever, leukopenia, eosinophilia (15%), liver damage (high liver function tests) and increased inflammatory markers (C-Reactive Protein – CRP - 220 mg/dL). DRESS syndrome secondary to piperacillin-tazobactam/vancomycin was suspected thus antimicrobials were suspended. The patient continued to clinically deteriorate, with marked cytopenia (1 120/uL leukocytes, 66 000/uL platelets, Hb 10 g/dL), increased ferritin (>10 000 ug/L) and hypertriglyceridemia (338 mg/dL). Ferritin levels were repeated and confirmed in two consecutive days. It was suspected of HLH syndrome secondary to an undiagnosed viral infection, which prompted DNA identification of EBV, Herpes Virus 6 and 7, CMV and Parvovirus B19. All tests were negative; thus, generalised inflammation associated with DRESS syndrome was considered the most probable cause of the HLH syndrome. Corticoid therapy was initiated with a clinically favourable response, with ferritin and CRP progressively decreasing and cytopenia resolving during the following weeks.

Discussion: Although there were strong suspicions of HLH syndrome, with diagnostic criteria from the American Society of Hematology Consensus on HLH Syndrome, bone marrow and soluble CD25 were not evaluated. This case report represents a plausible HLH syndrome secondary to DRESS syndrome.

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PERSISTENT LYMPHOCYTOSIS

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Introduction: Benign or reactive lymphocytosis with an absolute lymphocyte count is common in children and young adults and is usually related to infectious mononucleosis or other viral infections. On the other hand, benign lymphocytosis in adults is unusual. Persistent polyclonal B cell lymphocytosis (PPBL) is a rare and presumably not malignant lymphoproliferative disorder diagnosed predominantly in women. Clinical symptoms are nonspecific except for mild fatigue and patients are usually cigarette smokers. The peripheral blood examination shows in almost all cases atypical binucleated lymphocytes. Contrary to B-cell chronic lymphoproliferative disorders, peripheral B cells are polyclonal with kappa and lambda light-chain expression and no clonal rearrangement of immunoglobulin heavy chain genes is usually demonstrated.

Case Description: Female, 46 years old.

Personal history: smoker (62UMA), reflux esophagitis.

Referenced to Pulmonology Consultation, due to chest tightness and dyspnea for medium efforts with about one year evolution. She also referred asthenia and intermittent wheezing. There were requested several complementary diagnostic tests including clinical analysis. A peripheral blood smear was performed and the presence of a subpopulation of lymphocytes with morphological characteristics that suggested clonality was verified. Clinical Pathologist suggested performing an immunophenotypic study of the peripheral blood lymphoid populations, which showed lymphocytosis of B-lymphocytes, with apparent polyclonal character.

Discussion: PPBL is characterised by persistent and stable lymphocytosis with binucleated lymphocytes on peripheral blood smears. According to current literature, the B-cell proliferation pool can favor the emergence of subclones carrying oncogenic mutations, some of which may develop years later into subsequent lymphomas.

Conclusion: Combination between evaluation of the peripheral blood smear and immunophenotypic study allowed a correct diagnosis. Whether this syndrome represents a premalignant disease process or a polyclonal lymphocytosis remains unsettled, so we must monitor these patients along time.

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AGE-ADJUSTED D-DIMERS IN THE EMERGENCY DEPARTMENT SETTING

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Introduction: Pulmonary embolism (PE) is a life-threatening condition associated with a very high mortality. D-dimer (DD) testing is sensitive for thrombus formation and is used to rule out venous thromboembolism (VTE). DD has been shown to increase with age, which can cause a lower specificity in older patients, hence the growing recommendation of the use of age-adjusted DD (DDa) in patients over 50 years of age.

Objectives: This study aims to evaluate the use of a DDa threshold in our emergency department (ED) and determine if there are any benefits in comparison to the use of regular DD testing.

Materials and Methods: A retrospective study was conducted through the consultation of patients' laboratory, clinical, and radiological reports of all ED visits in which DD were tested, from patients with at least 50 years, in the period from 1 to 7 of November 2019. A total of 54 patients were included in this study, 57.4% were females and the average age was 72.3 ± 3.2 years old. DDa's threshold was calculated (patient's age x $10\mu\text{g/L}$), and so were the Wells' Criteria (WC) and Geneva Score (GS) to obtain an estimated clinical pretest probability (PTP) of PE.

Results: 21 patients (38.8%) had positive DD ($>500\mu\text{g/L}$), from which 13 had positive DDa (DD $>$ DDa), 10 underwent CTA and 2 had positive PE findings, while 19 had other non-related pathological processes. 8 patients with positive DDa were classified with a moderate WC PTP (vs. 14 positive DD), and 11 were classified with a moderate GS PTP (vs. 16 positive DD). There were no high PTP cases. CTA testing was done in 8 patients with positive DDa (vs. 10 with positive DD), detecting both cases with positive PE findings.

Discussion and Conclusions: Applying a DDa threshold, we observed a smaller number of patients in higher PTP groups and who underwent CTA testing. These results suggest that the use of DDa could help the clinics decide which patients should follow complementary testing, and eventually reduce associated costs. A limitation of the study is the small sample of patients, further study with a larger sample is needed to corroborate these results.

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LUPUS ANTICOAGULANT TESTING – CHANGING OUR LABORATORY STRATEGY

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Lupus anticoagulant testing – Changing our laboratory strategy

Background: Lupus anticoagulants (LA) are a heterogeneous group of auto-antibodies that react primarily with protein antigens in the presence of an anionic phospholipid surface. Most clinically relevant LAs are directed against lipid-associated β_2 glycoprotein I. In the context of clot-based coagulation tests, LA can interfere with phospholipid dependent steps but this effect can be neutralized by adding higher concentrations of phospholipids. Progress on the standardization for exploring the use of antiphospholipid antibodies present in samples as guidelines, were published.

Aim: This study aims to evaluate the benefit of using a second type of assay, by comparing the performance of two reagents to avoid diagnostic exclusion of some positive samples.

Materials and methods: 103 samples were evaluated in this study carried out using a ACLTOP 500 from IL. Screening and confirmation tests were performed for diluted Venom Viper Russel (dRVVT) as well as for Silica Clotting Time (SCT). To determine the reference window, 50 samples from healthy donors were used. Samples were considered positive for normalized ratios >1.2. We used Pearson correlation for concordance and correlation analysis between both methods.

Results: Data shows that 13,5% of the samples (n=14) were positive, of which, 5 (4.8%) were exclusively positive for dRVVT, 7 (6.8%) were positive for both, while 2 (1.9%) for SCT only, which would be treated as false negatives. The Pearson correlation coefficient $r = 0.682$, shows a moderate to low level of agreement reflecting a weak correlation between reagents. Thereby, there are no 100% sensitive reagents and the use of a single one, could lead to interpretive discrepancies.

Conclusion/Discussion: Since dRVVT has detected more positive samples, we propose to start with it, and if negative, proceed with SCT. Thereby, a single positive test leads to a positive result, naturally repeated after 12 weeks as suggested by guidelines. Therefore, is judged to be eligible for change in the routine settings of Lupus anticoagulant testing.

Bibliog: Moore GW, Maloney JC, de Jager N, et al. Application of different lupus anticoagulant diagnostic algorithms to the same assay data leads to interpretive discrepancies in some samples. *Res Pract Thromb Haemost.* 2017;1:62–68.

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EOSINOPHILIA CLONAL VS REACTIVE IN DXH800

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The normal eosinophil count ranges are 0.05-0.5x10⁹/L in peripheral blood and are derived from CD34+ hematopoietic precursor cell under the control of transcription factors, signal transducer and cytokines.

Blood eosinophilia is defined as an elevation of the eosinophil count, usually 0.5x10⁹/L or greater persisting for at least 6 months.

The causes of eosinophilia are numerous and are divided into three main categories: reactive (secondary), when result of a cytokine-mediated reactive phenomenon, clonal (primary) or idiopathic, depending on the respective presence or absence of a cytogenetic or molecular changes, such as tyrosine kinase gene fusions, typically involving the gene coding for platelet-derived factor receptor alpha (PDGFR α), platelet-derived factor receptor beta (PDGFR β) or fibroblast growth factor receptor 1 (FGFR1).

In our laboratory, the eosinophilia incidence is 2%, and our question is: analyzing the investigation parameters obtained due to VCSn (Volume, Conductivity and Scatter) technology of the DxH800 UniCel[®] Beckman Coulter is it possible to distinguish between reactive or clonal eosinophilia.

We performed 84 complete blood count and differential count, and identified 72 samples with eosinophilia and 8 samples control (without eosinophilia). Then we determined IgE values (UI/mL) of these samples to divided reactive or clonal eosinophilia. We evaluated the CD140a (PDGFR α) and CD140b (PDGFR β) expression of eosinophils in all samples. Finally, we performed to statistic analyze by means of one-way ANOVA test.

We observed that the absolute count of eosinophil is greater in clonal group with statically significance. Regarding conductivity of these eosinophils, we didn't observed difference between clonal and reactive eosinophilia. We observed that both reactive or clonal eosinophils have more granules than eosinophil of control group with $p < 0.05$. IgE values are greater in reactive group, as expected. The samples of clonal group have more expression of CD140a and CD140b, although without statically significance for mean intensity fluorescence (MIF).

In conclusion, the eosinophil count of clonal group is greater, more granular and has more expression of CD140a and CD140b.

We conclude, that by just analyzing the samples on DxH800 UniCel[®] Beckman Coulter it is possible to distinguish between clonal and reactive eosinophilia, through the investigation parameters, volume, conductivity and scatter.

CLINICAL CASE – FERROPENIC ANEMIA REFRACTORY TO THERAPEUTIC IN A PATIENT WITH HELICOBACTER PYLORI INFECTION

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Introduction: Iron deficiency is the most frequent cause of anemia. In Portugal, it is estimated that the prevalence of the disease is 20% with the majority of patients being asymptomatic. Helicobacter pylori (H. pylori) infection is probably the most common worldwide and, in Portugal the prevalence of infected individuals is particularly high. There are several published studies that link the two pathologies. Bacterial infection induces protein sequestration of iron, decreasing its absorption. An inflammatory response is generated, which, through the action of hepcidin, induces the destruction of iron-carrying proteins and decreases the production of ascorbic acid, a catalyst for the conversion of ferric to ferrous iron.

Objective: The authors present a clinical case of ferropenic anemia refractory to treatment, in a patient with an infection with H. pylori.

Clinical Case: A 57-year-old autonomous woman with an irrelevant background goes to a gastroenterology appointment for asthenia, epigastric pain, heartburn, anorexia and dyspnea on exertion, with six months of evolution. At the physical examination, the skin and mucous membranes are discolored and jaundiced. Analytically presents with microcytic and hypochromic anemia and anisopoikilocytosis. Serum iron and ferritin were decreased while transferrin saturation was increased. Normal vitamin B12 and folic acid were found. C-reactive protein was negative. The patient was diagnosed with ferropenic anemia and started treatment with oral iron for three months. In the post-therapeutic evaluation, the patient is better clinically, but maintains the analytical changes of the diagnosis. More complementary diagnostic tests were performed, one of which was fundamental. In the esophagus-gastro-duodenoscopy patient presented an alteration compatible with chronic gastritis with a positive biopsy for H. pylori. The patient undergoes treatment to eradicate the bacteria, maintaining oral iron therapy and obtains significant analytical improvements, with normalization of hemoglobin and iron metabolism parameters.

Discussion / Conclusion: This clinical case reflects the various studies that have been carried out in this area regarding H. pylori infection as one of the causes of ferropenic anemia and the treatment of the two pathologies must be combined.

CLINICAL PATHOLOGISTS ON WATCH: DIAGNOSIS OF ACUTE MYELOID LEUKEMIA IN A CHRONIC LYMPHOCYTIC LEUKEMIA PATIENT

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Introduction: Acute myeloid leukemia (AML) rarely develops in patients with other cancers. AML and myelodysplastic syndrome have also been associated with therapy in chronic lymphocytic leukemia (CLL) patients with an incidence of <1%. There are few cases describing CLL patients which developed AML 2 weeks to 4 years after treatment with cyclophosphamide and/or rituximab and/or vincristine and/or prednisone.

Case description: An 80-year-old man presented to emergency room (ER) in November, 2019 with an acute-onset of right knee pain and oedema, general weakness and fever. Recent past medical history included: prostate cancer diagnosed in January, 2018 and treated with leuprolide; clear cell renal cell carcinoma diagnosed in June, 2018 and treated with nephrectomy; small lymphocytic lymphoma diagnosed in June, 2018 and treated with 6 cycles of complex chemotherapy (rituximab + cyclophosphamide + vincristine + prednisolone), initially responded well, but progressed to CLL in October, 2018 (analytically presented hemoglobin: 7.8 g/dL, white blood cells 22.14 x 10⁹/L with lymphocytosis, platelets 80 x 10⁹/L) and started Ibrutinib, response was positive prior to presentation.

Physical examination in ER revealed skin pallor and hepatosplenomegaly. Initial peripheral blood (PB) count and smears showed critical anemia, thrombocytopenia, and leukocytosis (hemoglobin: 5.2 g/dL, platelets 75 x 10⁹/L, white blood cells 93.73 x 10⁹/L) with two distinct populations suggesting abnormality: small cells corresponding to mature lymphocytes (83%) and large cells corresponding to blast cells (13%), with Gumprecht cells also present. In four weeks the blast cells proportion raised to 37%.

PB immunophenotypic analysis confirmed morphological findings. CLL diagnosis was confirmed by demonstrating the expression of mature B-cell markers (CD19+, CD20dim) and CD5+, CD10-. Blast cells expressed myeloid markers (CD33+, MPO+) and "AML not otherwise specified" was diagnosed.

Discussion and conclusions: Anemia and thrombocytopenia development in cancer patients may be interpreted as disease progression or iatrogenic effects related to aggressive treatment, leading to delayed diagnosis. The present case highlights the need of careful PB smear revision, especially in CLL patients where the automatic analysis equipment routinely produces abnormal leucocytes presence alerts.

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COMPLETE BLOOD COUNT - CBC - O ALGORITHM: USEFUL IN DAILY CLINICAL PRACTICE

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Introduction: One of the main objectives of laboratorial clinical analysis is to provide accurate results as fast as possible; 70% of clinical decisions are based on laboratory results.

The CBC is a parameter frequently requested and interferences in the samples may occur (presence of cryoglobulins, hemolysis or lipemia). These lead not only to incorrect results but also require doing additional procedures specifically heating in the case of cryoglobulin's presence or washing blood cells for hemolysis and lipemia.

Thus, there was a need for the equipments to be able to apply more than one methodology when determining the same parameters - like the Sysmex XN series hemocytometer, that has the possibility of determining Red Blood Cell Count (RBC) both by impedance and optical methods. The CBC-O algorithm (CBC-Oa) applied by the E-IPU Sysmex software is triggered whenever MCHC >37g/dL, being able to detect and identify the interferer, correcting it through an optical methodology at 41°C.

Case report 1: 1st CBC determination: HGB 20.0 g/dL; RBC 5.0x10¹²/L; HCT 42.4%; MCV 74.0 fL; MCH 34.9pg; MCHC 47.2 g/dL.

Macroscopically: lipemic sample.

CBC-Oa correction: HGB 15.1 g/dL; MCH 26.4 pg; MCHC 35.6 g/dL.

This is a case of Hg overestimation due to lipemia, in which CBC-O prevented the washing of blood cells to obtain HGB levels.

Case report 2: 1st CBC determination: HGB 7.7g/dL; RBC 1.43x10¹²/L; HCT 11.5%; MCV 80.4 fL; MCH 53.8 pg; MCHC 67.0 g/dL.

CBC-Oa correction: RBC 3.77x10¹²/L; HCT 25.8%; MCV 68.5 fL; MCH 20.4 pg; MCHC 29.8 g/dL.

This is a case of interference in the RBC and MCV count due to the presence of cryoglobulins, in which the CBC-O prevented the sample from being heated in an oven at 37°C for 15 minutes.

Case report 3: 1st CBC determination: HGB 8.0g/dL; RBC 2.35x10¹²/L; HCT 21.1%; MCV 89.8fL; MCH 34.0 pg; MCHC 37.9 g/dL.

CBC-Oa correction: HGB 7.2g/dL; MCH 30.6 pg; MCHC 34.1 g/dL.

Macroscopically hemolyzed sample; biochemical parameters such as K⁺ and LDH, suggestive of in vivo hemolysis.

This is a case of interference in the HGB measurement due to the existence of in vivo hemolysis, in which the CBC-O allowed obtaining its correct value.

Conclusion: The application of the CBC-O algorithm is an asset in daily practice in a clinical laboratory, as it avoids more time-consuming procedures and the overload of technical work.

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URINE EOSINOPHILS IN THE DIAGNOSIS OF ACUTE INTERSTITIAL NEPHRITIS

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Introduction: Acute Interstitial Nephritis (AIN) is characterized by a diffuse infiltrate of inflammatory cells in the kidney interstitium. Urine Eosinophils (UE) has been used for a long time as a marker of AIN, however recent studies advise against its use due to low sensitivity and specificity. The Gold-Standard for diagnosis is kidney biopsy.

Objectives: The objective is to characterize the requests for UE in a hospital, over a 2-year period and to study its relationship with the diagnosis of AIN.

Materials and Methods: We performed a retrospective study of UE requests received at the Clinical Pathology Laboratory of a Hospital, from 2018 to 2019.

The first test for each patient was included in the study. The presence of kidney biopsy (performed with a maximum interval of 1 month of UE) and the final diagnosis of the episode were verified in the clinical process.

UE were considered to be positive when eosinophils were at least 1% of total urinary leukocytes.

Results: There were 99 patients included in the study. Of the total UE tests, there were 67 with a negative result and 32 with a positive result.

Of the cases of clinical diagnosis of AIN, 46.7% had negative UE and 53.3% had a positive test. In cases where the diagnosis of NIA was not made, 71.4% had a negative test and 28.6% a positive test. With the exception of contrast nephropathy, in all other diagnoses there were cases of positive UE, namely in prerenal Acute Kidney Injury (AKI) 23.8% of patients had positive UE, in post-renal AKI 2 in 3 patients and in infection and glomerular disease 2 in 5.

There were 9 patients with renal biopsy results. Of these, 5 had positive UE: 2 cases with histology result compatible with AIN, 1 case of glomerular disease and 1 case of neoplasia. In 4 patients with renal biopsy, UE was negative, with 1 case of NIA, 1 case of diffuse glomerulosclerosis and 1 case of amyloidosis.

Conclusion: Eosinophiluria were found in a variety of kidney pathologies and its absence does not rule out the diagnosis of AIN. It is an insensitive and unspecific test that can lead to delays in diagnosis and unnecessary treatments.

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HEMOGLOBINOPATHY SC-CLINICAL CASE

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Introduction: Hemoglobinopathy SC it is a variant of sickle disease, the second most common in the world after SS genotype. It presents a less severe clinical course, compared to sickle cell anaemia and occurs at a more advanced age, however, acute complications in the target organs may occur with varying intensity.

Clinical case: 52-year-old male, Brazilian, turned to the Emergency Department with intense abdominal pain in the right hypochondrium with four days of evolution. He referred anorexia, weight loss, asthenia and pain crises of varying intensity especially in the lower limbs. Antecedents-Chronic anaemia, vesicular lithiasis, pulmonary tuberculosis, bilateral hypoacusis, progressive vision loss. Refers his father (deceased) and brother with chronic anaemia. Physical exam - Abdomen: Right hypochondrium painful to the touch, positive vesicular Murphy's. He presented the following analytical results: Haemoglobin (Hb) 11 g/dl, MCV 70.6 fl, MCH 25.5 pg, reticulocytes 3.18 % and ferritin 452.72 µ/ml. Abdominal CT scan-Coarse calcifications in splenic cavity. He was admitted for an urgent cholecystectomy. Given the strong suspicion of hemoglobinopathy, its etiology started.

Result/Discussion: Peripheral blood morphology: Anisocytosis, microcytosis, hypochromia, poikilocytosis, many target cells, rare drepanocytes, rare haemoglobin crystals, boat-shaped cells, rare erythroblasts. Positive solubility test. The HPLC (High Performance Liquid Chromatography) Technique. Electrophoresis at alkaline pH, a variant of Hb was detected migrating in the HbS area and an abnormal band migrating in the HbA2 position. Electrophoresis at acid pH, made it possible to separate HbS and HbC from other variants that migrated in the same position, when submitted to electrophoresis at alkaline pH. Genetic study- Changes c.20>T p (Glu-7Val) and c.19G>A p. (Glu-7Lys). Genotype at cDNA level, HBB- c. [19G>A]; [20A >T].

Conclusion: Double Heterozygosity SC. The patient was referred for Haematology, Ophthalmology and Pulmonology appointments, showing improvement of the clinical and analytical signs. This clinical case highlights the importance of the laboratory in the study of hemoglobinopathies, as well as the role of the clinical pathologist in the diagnostic clarification process.

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BLASTIC MANTLE CELL LYMPHOMA – CLINIC CASE

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Introduction: Mantle cell lymphoma (MCL) is a rare lymphoma and is considered an aggressive and incurable disease. However some indolent presentations have been described. The more aggressive variants are the blastoid and the pleomorphic ones. In the blastoid variant the cells resemble lymphoblasts with dispersed chromatin and a high mitotic rate. MCL usually presents with adenopathies, with some rare cases presenting as peripheral blood leukemia.

Clinic case: We present a case of a 87 years old man who presented in an periferic hospital with anorexia, weight loss and progressive asthenia for the last 3 months. Physical examination showed only a splenomegaly of 10 cm. No enlarged lymph nodes were palpable. The first laboratory tests showed an anemia with an hemoglobin of 10 gr/dL, a leucocytosis of 127 000 cells/ μ L with 41% of these as lymphocytes on automated cell counter. Morphologically, an uncounted number of blasts cells was described. The patient was sent to our hospital for further characterization of his disease. The blood tests were repeated and showed the same abnormalities, with no other significant lab results. An initial blood smear was observed and described as having 85% blast cells. A second peripheral blood smear was then observed in the hematology laboratory by another senior hematologist who described 95% lymphoid cells compatible with lymphoma morphology. A flow cytometry immunophenotyping analysis was compatible with Non-Hodgkin's MCL and a diagnosis was made. A sample was sent to cytogenetic analysis of t(11;14) IgH/CCND1 which came as positive.

Conclusion: This is a case of one of the rare patients who has a leukemia presentation of MCL. It shows both the importance of the blood smears for this diagnosis, and its characterization as blastic variant, and it's limitations as the definitive diagnosis was only made after the results for flow cytometry and cytogenetic analysis. Despite the correct description of one of our blood smears as lymphoma cells, further review of other acute lymphocytic leukemias showed very similar cells which calls to our attention the need to be careful when characterising cells with lymphoblast characteristics as definitive blasts.

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PROLONGED APTT - WHEN LABORATORY MAKES THE DIFFERENCE

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Introduction: Hemophilia A (factor VIII deficiency) is a severe congenital hemorrhagic disorder, affecting 1 in 5,000 to 10,000 male individuals. It presents forms of severity based on the level of plasma factor (<1% severe; 1 - 5% moderate; 6 – 30% discrete). Of the screening tests, only the activated partial thromboplastin time (aPTT) is prolonged.

Case summary: Newborn, 3 days old, with no relevant personal and family history. Went to the ER pediatric, one day after discharge from the nursery, with edema and pain in his right thigh, with approximately 30 hours of evolution (after the anti-HBV vaccine), also presenting with a recent hematoma of the right upper eyelid. The physical exam showed a sick and emaciated appearance, with skinfold. Jaundice of the skin and sclerotic. Axial hypotonia. Hematoma of the upper right eyelid. Exuberant swelling of the anterior aspect of the right thigh. Remaining examination without relevant changes. Laboratory tests showed normocytic / normochromic anemia and thrombocytosis; coagulation tests with an aPTT > 120 sec., which led the laboratory to carry out more coagulation studies. The result was consistent with deficiency of factors of the intrinsic pathway, thus being able to rule out other causes the elongate aPTT.

After successive ultrasounds at the level of the eyelid hematoma, there was a high risk of central bleeding, which was avoided with confirmation by the laboratory of the severe deficiency of Factor VIII, and the patient started treatment with recombinant Factor VIII, with rapid improvement, laboratorial and clinical.

Discussion: This case is intended to alert to the potential emergencies of Hemophilia A and the rapid intervention of the Emergency Laboratory in the performance of additional coagulation tests that contributed to an early diagnosis, which led to a timely targeted therapeutic approach, that was crucial to the patient's survival.

Take home message: In situations of unknown family history and / or without a history that suggest diagnosis of Hemophilia A, a severe hemorrhagic condition can result in an unfavorable outcome, in the absence of timely action by the laboratory. As documented in this case, the extreme value of the direct implications of laboratory diagnosis in the therapeutic approach is verified.

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COAGULATION: WHEN THERE IS MORE THAN ONE CULPRIT

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Introduction: The investigation of hemostasis disorders should be undertaken when a patient presents with a chronic hemorrhagic tendency, a basic coagulation test abnormality or an acute hemostatic failure with massive bleeding.

Coagulation disorders' are usually evaluated following an algorithm which determines the coagulation pathway that is abnormal and guides the following tests avoiding unnecessary testing and it's wrongful interpretation.

Case : We present the case of a 2 years-old boy who was admitted to the ER for an abdominal mass of unknown etiology with fever, abdominal pain and diarrhea. A pre-operative routine lab analysis revealed a prolonged Prothrombin Time (PT) of 21,7 seconds (s) and activated Partial Thromboplastin Time (aPTT) of 95,8 s.

This was followed by a mixing test of both PT and aPTT which was almost normal for PT with 13,6 s and aPTT remained prolonged at 62,9 s. Due to the discrepancy of these results, all coagulation factors were evaluated with only Factor VII being low (30%). Lupic anticoagulant also tested positive.

The study was repeated 12 weeks later with a new Factor VII result of 51%, fairly close to the inferior normal range, and a negative test for lupic anticoagulant, thus not confirming the Antiphospholipid Syndrome.

Discussion: This is an interesting case because it demonstrates that not all patients present with typical textbook coagulation abnormalities and how the usual management of these situations may not be the best. The inconsistency of the mixing tests aroused suspicion that no single abnormality was responsible for the results and this was confirmed by the subsequent studies.

On the other hand, it works as a reminder that the etiologic investigation of an hemostasis disorder should not be performed during an acute episode of illness for many of these factors may suffer variations in the acute ill patient and some non-specific transitory antibodies could interfere with the antiphospholipid testing. This may lead to a change of the results and a wrong diagnosis with inadequate therapeutic management.

RARE CASE OF THROMBOCYTOPENIA IN AN IMMUNOCOMPETENT YOUNG FEMALE ASSOCIATED WITH BONE MARROW PHAGOCYTOSIS

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Introduction: Macrophages are present in most of the tissues in the organism, including in the bone marrow (BM), where they are part of the hematopoietic system. Thrombocytopenia, defined as platelets count below to $150 \times 10^9/L$, is an important condition that can lead to clinical instability. The aetiology can be an increased destruction/consumption or a decreased production, sometimes requiring BM evaluation for differential diagnosis. We present a rare phenomenon of platelets' phagocytosis in the BM of a young healthy girl.

Clinical Case: 15 years old female patient with fever, adenomegalies and pancytopenia (Haemoglobin de 10,6g/gL; Platelets $56 \times 10^9/L$; Leukocytes $2,37 \times 10^9/L$; Neutrophils $0,79 \times 10^9/L$) was sent to this hospital for further study. The biochemical study showed an increased Aspartate Aminotransferase (64 U/L) and Lactate Dehydrogenase (1033 U/L) associated with hypocalcaemia, hypokalaemia, and 20,20 mg/L of CRP. Because of the suspicion of leukaemia, she was submitted to a BM evaluation. The BM smear showed a normal cellularity, with no apparent morphological alterations in the myeloid, erythroid and lymphoid cells. Megakaryocytes were present in a normal number and were bilobated. A rare find in the smear was the presence of macrophage cells phagocytosing numerous platelets. The immunophenotyping study of the BM showed no leukemic or malignant cells. Virologic screening tests were negative. The Autoimmunity screening showed Antinuclear antibody's positivity with a title of 1/320, anti-centriole antibody pattern. Anti-Sjogren's Syndrome-Related antigen A (Anti-SSA antibodies) and anti-Nuclear Ribonucleoprotein (Anti-RNP) were also positive.

Conclusion: The author's intention is to demonstrate a rare BM finding of platelets' phagocytosis by macrophages.

ANALYTICAL COMPARISON BETWEEN TWO HEMATOLOGICAL ANALYZER SYSTEMS: MINDRAY BC-6800 VS ADVIA 2120

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Objective: Compare the Mindray BC-6800 and Advia 2120 instruments by analysing the results of parameters of cell blood count in patient hematological samples.

Material and Methods: 134 patient samples were analysed for Leukocytes (WBC), Erythrocytes (RBC), Hemoglobin (Hb), Platelets (PLT) and Hematocrit (HCT).

The two groups of test results were tested by t test, using MedCalc Software. The difference between the two instruments was compared.

Passing-Bablok regression method was used to analyse the results of the two instruments. The results of WBC, RBC, Hb, PLT, and HCT were determined by Advia 2120 (y), and Mindray BC-6800 (x). A linear regression analysis was performed to calculate the regression equation. The intercept is a measure of the systematic difference between the two instruments. The slope is a measure of the difference in the ratio between the two instruments. Passing-Bablok regression analysis evaluates the correlation between the two instruments. Pearson's test was used to obtain the correlation coefficient. When the correlation coefficient r is ≤ 0.35 , the correlation degree is low; when r is 0.36-0.67, the correlation degree is moderate; and when r is 0.68-1.00, the correlation degree is high.

The test results of the two instruments were evaluated in the MedCalc Software for Bland-Altman analysis, and the deviation map was drawn.

Results: The average difference between the two instruments results is not significant ($P > 0.05$, t test).

The regression analysis showed a linear correlation between the results of the two instruments. The 95% confidence interval for the intercept of the regression equation for each test item includes 0, except for HCT, and there is no systematic error between the two instruments. The 95% confidence interval of the slope contains 1 for all parameters except for WBC and Hb, and there is no proportional difference between the two instruments, r is $> 0,99$ for all parameters except PLT ($r= 0,966$), which showed that the correlation is good.

The Bland-Altman analysis showed that both instruments had more than 95% of the points within the 95% consistency limit except HCT (93.3%).

Conclusion: It can be considered that the two instruments have good correlation and consistency, and the two instruments can replace each other.

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PERFORMANCE EVALUATION OF THE MINDRAY BC-6800 AUTOMATED HEMATOLOGY ANALYSER

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Objective: Evaluate the analytical performance of the BC-6800 according with state of the art quality specifications. (1)

Materials and Methods: Samples used for evaluation were selected randomly from samples of the patients attending the laboratory.

Statistical analysis was performed with Microsoft Excel and MedCalc Software.

Background, intra-run imprecision, inter-run imprecision, linearity and carryover were evaluated.

Linearity for leukocytes and platelets was assessed by the conduction of seven dilutions: $\frac{1}{2}$; $\frac{1}{4}$; $\frac{1}{8}$; $\frac{1}{16}$; $\frac{1}{32}$; $\frac{1}{64}$; $\frac{1}{128}$. The linear equation and the correlation coefficient between theoretical and practical values were calculated.

Carryover was performed, in triplicate of a sample with high concentration of the analyte in question (H1, H2, H3), with a subsequent analysis in triplicate of a sample with low concentration (L1, L2, L3) of the same analyte.

Percentage carryover is calculated as follows: $((L1-L3)/(H3-L3))*100$.

Intra-run imprecision or repeatability - a sample with normal values, one with increased leukocyte values and a sample with increased platelet values were used. The samples were analysed 10 consecutive times and the mean, the standard deviation (SD) and the coefficient of variation (CV) were calculated for each parameter.

Inter-run imprecision or reproducibility was carried out by analysing sample results of internal quality control through three concentrations levels (low, normal and high) during 30 consecutive days. For each haematological parameter, the mean, SD and CV were calculated.

Results: If the results of background count meet the state of the art specifications.

Linearity was verified for leukocytes and platelets, and for both it presented an excellent correlation coefficient between theoretical and observed values (0.999) (state of the art specification $r > 0.99$).

The carryover for all the assessed parameters meet the state of the art specifications.

Intra-run imprecision and inter-run reproducibility meet the state of the art specifications.

Conclusion: The BC-6800 proved to be a hematology analyser with a very good analytical performance. The results obtained in this study indicate the reliability of parameters offered by this analyzer.

REMOTE AUDIT FOR QUALITY CONTROL EVALUATION IN FOUR CLINICAL LABORATORIES FOCUS IN HEMATOLOGIC PARAMETERS - PORTUGAL AND BRAZIL, 2018

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Introduction: The external audit is an evaluation of the clinical laboratories Quality Management System (QMS). Quality Control (QC) in clinical laboratory is an analytical error detection system.

Count Blood Cells (CBC) is one of the most frequently requested laboratory tests, and is the basis of numerous medical interventions, and therefore the transmission of reliable analytical results is crucial.

Objective: Evaluate the QMS of four clinical laboratories, by a remote audit, analyzing Internal Quality Control (IQC) and External Quality Control (EQC) results in hematologic parameters.

Methodology: The remote audit focused on 4 clinical laboratories, 3 Portuguese and 1 Brazilian, was according ISO NP EN ISO 15189:2014. An online questionnaire prepared using REDCap (Vanderbilt), and 4 excel files allowed to collect the IQC and EQC results of the analyzed parameters (hemoglobin, red blood cells, white blood cells and platelets). The questionnaire enabled the collection of information from QMS. From the IQC and EQC data obtained, CV (%), Bias (%) and Total Error (TE) (%) were calculated. All data referred to the year 2018.

Results: All laboratories have implemented QMS (1 certified, 1 accredited, 1 certified and accredited, and 1 in the certification process) and participate in different EQAS programs.

Two laboratories make their own calculations for the IQC control chart and the others use the values provided by the suppliers.

For hemoglobin the range for CV% was 0.68 to 1.84, the root mean quadratic of bias% was 2.10 (0.66–2.90) and TE% was 1.78 to 5.79.

For red blood cells the range for CV% was 0.84 to 1.85, the root mean quadratic of bias% was 4.93 (1.29–9.48) and TE% was 2.66 to 11.66.

For white blood cells the range for CV% was 1.61 to 5.26, the root mean quadratic of bias% was 5.65 (2.93–7.20) and TE% 6.81 to 15.86.

For platelets the range for CV% was 2.24 to 3.79, the root mean quadratic of bias% was 11.81 (4.22–18.81) and TE% 9.46 to 24.93.

Conclusion: The laboratories involved were motivated to collaborate and sent their quality results. The laboratories with a TE higher than admissible total error concerning Desirable Biological Variation Database specifications 2018 must make an effort to improve the performance of their laboratories results. The remote audit seems to be an appropriate tool to evaluate and monitor the indicators of QMS according to the normative reference.

HEPARIN-INDUCED THROMBOCYTOPENIA: A DIAGNOSIS TO KEEP IN MIND

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Introduction: Heparin-induced thrombocytopenia (HIT) is a prothrombotic drug-induced disorder mediated by platelet-activating IgG antibodies that target platelet factor 4/heparin complexes (PF4-H). Two types of HIT are described: type I (10-30%), a benign mild thrombocytopenia with spontaneous resolution and type II (0.2-7%), a more severe antibody-mediated thrombocytopenia with thrombotic risk. Diagnosis rests on clinical assessment of

disease probability (mostly based on the use of 4T score that evaluates thrombocytopenia, timing, thrombosis and likelihood of other causes of thrombocytopenia) and laboratory testing (determination of anti-PF4 antibodies). Management requires prompt cessation of heparin and initiation of an alternative anticoagulant to prevent thrombosis.

Clinical Case: We present the case of a 68-year-old man, with history of chronic alcohol abuse (ceased in 2008). He was transferred from another institution due to cardiogenic shock secondary to acute myocardial infarction, concomitantly with a respiratory infection. At admission he presented with Hb 10.6g/dL (13.0-17.5g/dL), MCV 81.2fL, MCH 28pg, platelets 211x10⁹/L (150-400x10⁹/L); PT 17.3sec (10-14.1sec), INR 1.43 and APTT 32.6sec (25.1 - 36.5sec). He was already on day 3 of therapy with enoxaparin 40mg i.d. Six days pos-admission (and nine days after anticoagulation's initiation) a progressive decline in platelet count began (nadir 33x10⁹/L, representing a fall of 85%), raising suspicion of HIT. The 4T score was 5, corresponding to an intermediate probability of HIT. Anti-PF4 antibodies were investigated through a latex immunoturbidimetric assay (HemosIL® HIT-Ab(PF4-H), Werfen®), revealing a positive result of 1.2U/mL (0-1 U/mL). Enoxaparin was suspended and a different anticoagulant therapy (Fondaparinux 2.5mg b.i.d.) was initiated. Gradual recovery and stabilization of platelet counts (although with mild sustained thrombocytopenia) were observed. There were no thrombotic events.

Conclusion: Diagnosis of HIT in older patients presenting with complex and severe pathologies can be arduous, since they often present numerous causes for thrombocytopenia. However, considering the high morbimortality associated with HIT, it is an essential differential diagnosis of thrombocytopenia to consider.

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BURKITT LYMPHOMA: CASE REPORT

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Introduction: Burkitt's lymphoma (BL) is an aggressive lymphoma with a high proliferative rate, derived from germinal center B cells, characterized by translocation and deregulation of the MYC gene on chromosome 8. There are three distinct clinical forms of BL: endemic, sporadic and immunodeficiency-associated. We report a case of sporadic form of BL with fulminant course.

Case Report: A 87-year-old man was admitted in Urgency Department in October 2019 because of a generalized erythematous rash with 1 week of evolution that progressed despite ongoing treatment for suspected drug hypersensitivity reaction. On admission, he was prostrate, feverish and hypotensive. He had a history of severe mitral valve insufficiency, pulmonary hypertension, and a nodular lesion in the right lower pulmonary lobe under investigation. Laboratory findings revealed mild anemia (Hb 11.5 g/dl), thrombocytopenia (35 x 10⁹/L), severe neutropenia (0.38 x 10⁹/L) and mild lymphocytosis (6.26 x 10⁹/L). The light microscopic examination of peripheral blood film revealed 29% of morphologically immature lymphoid cells, with nucleoli and a basophilic cytoplasm with small vacuoles, suggestive of lymphoma cells. Flow cytometry confirmed the presence of phenotypically abnormal intermediate sized monoclonal B cells, whose immunophenotype established the diagnosis of BL: CD19+, CD20+, CD10+, CD38+high, CD79+, CD81+high, HLA-DR+, surface kappa immunoglobulin light chains+, BCL2+low. Lymphoma cells were diploid and had a high proliferative index (two cell cycles identified, both with >10% of cells in S phase). At the time of diagnosis, patient was in spontaneous tumor lysis, with renal dysfunction and cholestasis (serum LDH 6545 U/L, uric acid 18.3 mg/dl, creatinine 2.61 mg/dl, total bilirubin 1.33 mg/dl, alkaline phosphatase 158 U/L and gamma glutamyl transpeptidase 256 U/L). Given the patient's age and poor general condition, which impeded a curative therapeutic strategy, and comfort measures were initiated. The patient died 48 hours from admission.

Conclusion: Burkitt's lymphoma is a rare aggressive disease with several clinical presentations. This case illustrates the fundamental role of the clinical pathologist in establishing the diagnosis, namely through cell morphology and immunophenotype.

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CENTRAL NERVOUS SYSTEM INVOLVEMENT IN ACUTE MYELOID LEUKEMIA IN PEDIATRIC AGES – CLINICAL PATHOLOGY IMPACT IN RISK STRATIFICATION

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Introduction: Leukemia represents 30% of all malignant pathologies in pediatric ages. Acute myeloid leukemia (AML) represents 15% of all leukemias. The traditional presentation starts with muscle pain, fever, cytopenia, adenomegalies and organomegalies. accordingly to bibliography, central nervous system is more rarely affected and appears in 5-20% of AMLs in childhood. The aim of this work is to present a less frequent clinical case so as to point out the importance of a systematic diagnosis and risk stratification.

Clinical Case: A 2 year old female child, presents to the emergency department of a peripheral hospital with right pre-auricular tumefaction and petechiae. The patient was treated with amoxicillin / clavulanic acid for 10 days. Analytically showed normocytic normochromic anemia, thrombocytopenia and 8% of circulating blasts. It was decided to transfer the patient to an oncological reference hospital for suspected acute leukemia. A bone marrow aspiration was performed showing a hypercellular marrow with 25,3% immature blast cells population morphologically compatible with AML, confirmed with flow cytometry (17,35% Myeloid blast cells, expressing 2 T-cell aberrant markers-CD4+ and CD7+). A lumbar puncture cytology showed blast cells, confirmed afterwards with flow cytometry, pointing to a CNS involvement. Molecular genetic tests showed a complex karyotype indicative of poor prognosis. Gene FLT3 was negative, as well as recurrent genetic abnormalities. Magnetic resonance imaging was inconclusive about the etiology of the tumefaction, infectious or tumoral infiltration. Due to this clinical presentation a chemotherapy protocol for AML, with CNS invasion, was initiated.

Discussion: Lumbar puncture, cytologic exam and Immunophenotypic characterization of cerebrospinal fluid are the main approaches to leukemia patients with neurologic complications. Early diagnosis as well as fast and effective treatment generally improves the patient outcome.

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ADJUSTING PRE-ANALYTICAL CRITERIA IN ORDER TO MINIMIZE IMPACT ON CANCER PATIENTS

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Introduction: Cancer patients undergoing chemotherapy often present fragile veins, small in diameter, and difficult to anchor. A high prevalence of antiphospholipid antibodies in cancer patients often obligates two citrate tube collection when coagulation testing and Lupus Anticoagulant (LA) detection are required, being strenuous and tiresome for phlebotomists and patients.

Pre-analytical conditions regarding patient circumstances should be evaluated.

Objective: Assessment of prothrombin time (PT) and activated partial thromboplastin time (aPTT) using the same sample for LA.

Material and Methods: Platelet count (PLT), PT and PTT were performed in 80 patients in both plasma and platelet-poor plasma (PPP) using a Beckman Coulter LH750 for PLT, ACL TOP 500 with HemosIL® Recombiplastin 2G for PT, HemosIL® Synthasil for aPTT, HemosIL® dRVVT and HemosIL® SCT for LA.

Peripheral blood collection was performed for 2,8% sodium citrate tubes (1/9 sodium citrate vs blood).

Plasma was obtained with single centrifugation and PPP with double centrifugation at 1500g for 15 minutes with a centrifuge brake set off.

Three groups were created in both plasma and PPP samples: total, positive LA and negative LA.

Student's t-test was used for group comparison.

Results: No significant statistical differences were obtained for PT or aPTT results on corresponding samples on either group evaluated.

PT comparison for total, positive LA and negative LA were $\rho=0.4732$, $\rho=0.7043$ and $\rho=0.4006$ respectively. aPTT comparison presented $\rho=0.1990$, $\rho=0.2947$ and $\rho=0.0921$ for the same groups.

PT presented a minimum and a maximum of 9.8" and 23.8" in plasma, and 9.7" and 23.0" in PPP. Minimum and maximum aPTT values were 20.8", 126.2" in plasma and 21.5", 137,0" in PPP.

Platelet counts were evidently different between plasma and PPP samples evaluated ($\rho=4.9617 \times 10^{-12}$), with a minimum of $5 \times 10^3/\mu\text{L}$, maximum of $296 \times 10^3/\mu\text{L}$ in plasma and $1 \times 10^3/\mu\text{L}$, $80 \times 10^3/\mu\text{L}$ in PPP samples.

Conclusions: Cancer patients face hard physical conditions, hence pre-analytical criteria are crucial in minimizing the negative impact of blood sampling.

No differences were observed for PT and aPTT results between plasma and PPP.

This allows the determination of PT and aPTT in PPP when LA evaluation is also required, with benefits for the patient and less sample manipulation by technicians.

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THE MONITORIZATION OF ANTIPLATELET THERAPY IN STROKE PATIENTS – TWO CASE REPORTS ANALYSIS

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Introduction: Portugal is a leading country in stroke mortality and secondary incapacity in Western Europe. Current guidelines for secondary prevention of stroke recommend antiplatelet therapy and its monitorization in clinical practice should be common place. Platelet inhibitors such as acetylsalicylic acid (ASA) and clopidogrel are used to minimize the formation of thrombi but not all patients respond well with up to 25% of patients responding inadequately to clopidogrel. We present two case reports that show the results of platelet function tests (PFT) - platelet aggregation curves in monitoring of antiplatelet therapy. The PFT were performed on Multiplate® Analyser, Roche®.

Case Report: The first case is a 66 year-old man with diagnosis of transient ischemic attack and imagiologic evidence of lacunar stroke syndrome; the patient was monitored for double antiplatelet therapy with ASA and clopidogrel which revealed decreased levels in PFT of ADP (adenosine diphosphate) (6.4 $\mu\text{M}/\text{mL}$) 20U, Collagen (3.2 $\mu\text{g}/\text{mL}$) 70U and Arachidonic acid (0.5 mM) 8U; evidence of effective therapeutic range. The second case is a 47 year-old woman with the diagnosis of right middle cerebral artery ischemic stroke one year ago, at this moment on single antiplatelet therapy with clopidogrel in which the PFT revealed only slightly decreased levels of ADP 53U. **Discussion and Conclusion:** Patients with high residual on-treatment reactivity have been shown to be at increased risk of recurrent ischemic events. When identified, the increased risk can be reduced by switching these patients to other antiplatelet therapies. This guidance and personalization of antiplatelet therapy can only be achieved through monitoring of PFT - platelet aggregation curves. The efficiency of this laboratory method to monitor the antiaggregant inhibitors and help prevent thrombotic ischemic recurrences should be made aware and become well established among clinicians.

ESSENTIAL MYELOGRAM IN A PANCYTOPENIA CASE

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Introduction: The bone marrow is frequently involved in a variety of cases presenting with hematological and non-hematological disorders. A myelogram is a differential study of the cellular elements present in bone marrow, usually made on material obtained by sternal biopsy, which can help in the diagnosis of such disorders.

Clinical case: A 58 year old male presents himself at the Emergency Department (ED) of a tertiary care teaching hospital, with complaints of fatigue, anorexia and a 6 Kg weight loss in 6 weeks. No prior relevant clinical history.

The analytical evaluation conducted in the ED revealed pancytopenia, with 5.8 g/dL hemoglobin, 2.08×10^9 /L white blood count and 137×10^9 /L platelets and a high concentration of lactate dehydrogenase, 9700 U/L.

The peripheral blood smear revealed erythrocyte anisocytosis and poikilocytosis, and the presence of hypersegmented neutrophils.

The analytical evaluation and the peripheral blood smear suggested two possible clinical diagnosis: rapidly progressive lymphoproliferative disease or acute leukemia with eventual tumoral lysis syndrome.

To better study the case, the patient was admitted at the hospital and a myelogram obtained by sternal biopsy was done. The myelogram revealed a moderate hypercellular bone marrow, with inverted myeloid/erythroid ratio and findings characteristic of megaloblastic anemia, namely large megakaryocytes. Based on this elements, the patient was diagnosed with megaloblastic anemia.

Laboratory assessment of serum Vitamin B12 was later performed, and confirmed the diagnosis.

Discussion: The patient's case study required several tests to be performed. The necessity to perform a myelogram emerged from the rapidly progressive severity of the symptoms and the need to diagnose the cause of bone marrow failure. Signs of megaloblastosis were observed and were fundamental in the diagnosis of megaloblastic anemia.

In most cases the myelogram only confirms the megaloblastic anemia diagnosis and it's rarely needed. In this case, however, it was essential because both the initial diagnosis considered were hemato-oncological diseases and it was urgent to have a final diagnosis so the proper treatment could be initiated.