GERMANO DE SOUSA
CENTRO DE MEDICINA LABORATORIAL

The Laboratory at the Clinical Practice Center

CLINICAL CASE BOOK

— 2025 —

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Germano de Sousa Laboratory Medicine Center
The Laboratory at the Heart of Clinical Practice
Clinical Case Book - 2025

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INTRODUCTORY NOTE

Medicine is currently undergoing a period of profound transformation. Laboratory research, grounded in advances in biology, is experiencing significant growth.
Molecular and cellular technology has ceased to be merely a diagnostic tool and has assumed a central role in clinical practice.

In this context, the clinical pathologist has an irreplaceable role. More than just a behind-the-scenes specialist, he is...
A true interpreter of the language of disease. They translate the complexity of laboratory results into useful and precise information, indispensable to the clinician in decision-making. Their work is not limited to the technical accuracy of a result: it extends to collaborative reflection, dialogue with fellow assistants, and support in defining the best therapeutic strategies.

It is this vision that inspires the activity of Germano de Sousa Laboratory Medicine Center. Here, science and clinical practice.
They walk side by side, united by the commitment to offer solid diagnoses and personalized solutions for each patient. Our Clinical Pathology Laboratory is, above all, a space for sharing and collaboration, where different areas of expertise meet for the benefit of health.

The clinical cases gathered in this book are a clear expression of this mission. Each example reflects not only technical rigor, but also the close relationship between clinical pathologists and attending physicians, demonstrating how interdisciplinarity translates into better care and more humane follow-up. May these pages serve as inspiration for all healthcare professionals who believe, as we do, that medicine is built on dialogue between science, experience, and dedication to people.

Clinical Director
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CASE N.º 1

Bacteremia Streptococcus gallolyticus in a patient with aortic endocarditis and lumbar spondylodiscitis

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ABSTRACT

The authors present a case of a rare association between aortic endocarditis caused by *Streptococcus gallolyticus*, *subspecies gallolyticus*, and lumbar spondylodiscitis.

In January 2018, a 78-year-old female patient was admitted to investigate a condition, observed since November 2017, involving intermittent fever and persistent, incapacitating lower back pain. Initial analytical parameters revealed a significant inflammatory component, and the most likely diagnosis was initially assumed to be a non-infectious inflammatory disease. On the 10th day of hospitalization, a strain of *Streptococcus gallolyticus/bovis* serotype 1 was isolated from several blood cultures, and the hypothesis of endocarditis was confirmed by transesophageal echocardiography (aortic valve vegetation).

Given the persistence of low back pain, a new magnetic resonance imaging (MRI) scan was performed (four weeks after the first), that revealed signs of spondylodiscitis with an associated abscess. A colonoscopy was performed to exclude the presence of colorectal carcinoma, which led to the detection of a large polyp.

The identification of the agent in blood cultures led to both the colonoscopy and the investigation of the underlying endocarditis, allowing the removal of a large polyp and the diagnosis of aortic valve disease.

INTRODUCTION

Several cases described in the literature report an association between infection by *Streptococcus gallolyticus* subspecies *gallolyticus*, colorectal tumors, and infective endocarditis. However, combined infection with this microorganism involving colorectal pathology and spondylodiscitis is rare.
This case illustrates the diagnostic workup following the unexpected identification of the bacterium, leading to targeted antibiotic therapy and a favorable clinical outcome.

CASE

A 78-year-old female patient was admitted in January 2018 to investigate intermittent fever accompanied by chills and intense, debilitating lower back pain, the latter having been present since November 2017. Previous medical records highlight the following comorbidities: hypertension, renal insufficiency, and chronic bronchitis. She has a history of previous surgical procedures involving lumbar spine instrumentation and cervical compression syndrome, performed 20 and 7 years prior, respectively.
Initial analytical parameters revealed mild renal insufficiency (uremia 86 mg/dL and creatinine 1.64 mg/dL) and a marked inflammatory component, with an erythrocyte sedimentation rate of 115 mm/hour, CRP of 8.7 mg/dL, and protein electrophoresis showing an inflammatory pattern. Lumbar magnetic resonance imaging showed no signs suggestive of spondylodiscitis or other inflammatory processes, and electromyography (EMG) ruled out significant neurological damage to the lower limb. In this context, it was presumed that this clinical picture was probably a non-infectious inflammatory disease, and analgesic therapy, corticosteroid therapy, and rehabilitation were initiated. On the 10th day of hospitalization, a strain of *Streptococcus gallolyticus/bovis Serotype* I was detected in four blood cultures (Quilaban® BACTEC 9500®), identified using the MALDI-TOF methodology (BioMérieux VITEK MS®). Endocarditis was initially suspected, although the transthoracic echocardiogram was negative at the time. The following day, a transesophageal echocardiogram confirmed aortic valve endocarditis, with moderate regurgitation and possible mitral valve involvement (image suggestive of vegetation on the mitral valve). Antibiotic therapy with ceftriaxone was initiated immediately.
A colonoscopy was performed to rule out colorectal tumors, and a large polyp without ulcerative lesions was detected.
Due to persistent, worsening lower back pain, accompanied by gait impairment and elevated inflammatory parameters (ESR and CRP) in the following days, a new MRI of the lumbar spine was performed, revealing spondylodiscitis in the L5-S1 segments with an associated epidural space abscess, progressing to the L4-L5 levels. Inflammatory/infectious changes were also observed in the L5-S1 facet joints. Antibiotic therapy with vancomycin and meropenem was initiated, later changed to linezolid and meropenem, for four weeks. Surgical drainage was not indicated.
Following antibiotic treatment, the patient progressed favorably, with remission of back pain and impaired walking. Follow-up transesophageal echocardiography revealed residual aortic valve vegetation without insufficiency significant valvular and complete regression of the vegetation of Mitral valve. The lumbar MRI showed substantial.

INVESTIGATION

Blood count: normocytic and normochromic anemia;
Liver function: no relevant alterations;
Renal function: elevated urea and creatinine;
Increased CRP and sedimentation rate;
Blood cultures: positive for *Streptococcus gallolyticus spp gallolyticus*;
Abdominal ultrasound: no significant changes;
Renal ultrasound: slight asymmetry between the right kidney (RD) and left kidney (RE);
Transthoracic cardiac ultrasound: no valvular vegetation;
Electromyography with conduction velocity: no neurological abnormalities;
Lumbar spine magnetic resonance imaging: spondylodiscitis process with abscess;
Colonoscopy: surgical removal of large polyps;
Gynecological and bladder ultrasound: no relevant alterations;
Rheumatological serologies negative. Rheumatoid factor and negative reaction of *Waaler-Rose*;
Serum immunoglobulins: within reference values; Serum immunoelectrophoresis: no alterations;
Transesophageal cardiac ultrasound: showed endocarditis of the aortic valve and mitral valve involvement;
Fractions C3 and C4 of the complement without significant changes.

TREATMENT

Antibiotic therapy with vancomycin and meropenem, later changed to linezolid and meropenem, for four weeks.

RESULTS AND MONITORING

Following antibiotic treatment, the patient progressed favorably, with remission of back pain and gait impairment. Follow-up transesophageal echocardiography revealed residual aortic valve vegetation without significant valvular insufficiency and complete regression of mitral valve vegetation. Lumbar magnetic resonance imaging showed substantial improvement, and the patient was discharged after two months of hospitalization.

DISCUSSION

The *Streptococcus gallolyticus* (previously *Streptococcus bovis* [2]), belongs to Group D of *Streptococcus*, and is a Gram-positive, catalase-negative facultative anaerobic agent that is part of the digestive tract flora in 2.5-15% [2] of healthy people.
There are several species of *Streptococcus* from Group D, the most important being *Streptococcus gallolyticus* subspecies *gallolyticus* (previously *Streptococcus bovis I*), *Streptococcus gallolyticus subspecies pasteurianus* (previously *Streptococcus bovis II / 2*) and *Streptococcus infantarius* (previously *Streptococcus bovis II / 1*), which includes two subspecies: *coli* and *infantry*.
The Streptococcus gallolyticus It is an important cause of bacteremia and infective endocarditis in adults [1]. Other clinical presentations, such as infections of the urinary tract infections, central nervous system infections, or vertebral infections. The well-documented association between infecti-

ve endocarditis and *S. gallolyticus* and colorectal tumors justify the clinical indication for colonoscopy, when the S. gallolyticus spp gallolyticus It is identified in cases of bacteremia or endocarditis. Despite the existence of several articles on this association, the topic remains controversial; 25-80% of patients with bacteremia due to *S. gallolyticus* they have concomitant colorectal tumors, and 18-62% of patients with endocarditis due to... *S. gallolyticus* they present with colorectal tumors, the vast majority being *S. gallolyticus spp gallolyticus*. [2] Infective endocarditis due to *S. gallolyticus* It mainly affects elderly patients with comorbidities. The presentation is frequently subacute, multivalvular, and with vegetation. However, 43-72% of affected patients do not have valvular heart disease [1].

In this case, laboratory identification of *S. gallotyticus spp gallolyticus*, The findings in four blood cultures led to the findings on the echocardiogram, first transthoracic and later transesophageal, revealing infective endocarditis (vegetative lesions on the aortic valve and mitral valve involvement), and to the colonoscopy with resection of a polyp (benign tumor).

The persistence of painful and debilitating lumbar complaints, with fever and elevated inflammatory markers, even after the start of antibiotic therapy, led to an investigation of the lumbar spine imaging, showing spondylodiscitis with abscess, treated with specific antibiotic therapy. As the patient did not have a formal indication for surgical drainage of the abscess, no culture studies of the etiological agent were performed. However, the clinical resolution and favorable imaging, after antibiotic therapy targeted to *S. gallolyticus spp gallolyticus*, This led us to consider this agent as a likely cause of the spinal injuries.

POINTS TO HIGHLIGHT

- The association between *Streptococcus gallolyticus* subspecies *gallolyticus*, Aortic endocarditis and lumbar spondylodiscitis are rare.
- A bacteriemia por *Streptococcus gallolyticus* subespécie *gallolyticus*, deve levar à investigação da endocardite subjacente.
- A bacteriemia por *Streptococcus gallolyticus* subespécie *gallolyticus* ou endocardite, devem levar à colonoscopia [3].

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CASE N.º 2

May-Hegglin anomaly

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ABSTRACT

We report the case of a 23-year-old woman from Timor, referred to the Haematology consultation for chronic asymptomatic thrombocytopenia, diagnosed in 2007. The peripheral blood smear revealed giant platelets and basophilic cytoplasmic inclusions in the neutrophils (Döhle bodies). Platelet counts ranged from 32,000 to 89,000/L, depending on the method used, suggesting an interference of platelet morphology in the count. Platelet function revealed slight changes attributable to thrombocytopenia. Normal renal and hepatic function; negative antiplatelet antibodies. These findings have led to clinical suspicion of May-Hegglin anomaly, a rare entity within the spectrum of diseases related to mutations in the MYH9 gene, characterised by macrothrombocytopaenia and leukocyte inclusions, which generally has a benign course, not requiring specific treatment.

INTRODUCTION

The Anomaly of *May-Hegglin*, the Syndrome *Sebastian* and the Syndrome *Fechtner* These are rare autosomal dominant hereditary diseases characterized by the triad of giant platelets, thrombocytopenia and cytoplasmic inclusions in granulocytes (Döhle body type) (1). These diseases result from a mutation in the MYH9 gene, non-muscle myosin heavy chain class IIA (NMMHCA) gene, on chromosome 22q12.3--q13.2 [2].

Macrothrombocytopenia is secondary to a defect in megakaryocytic maturation and fragmentation, which does not alter platelet function. Inclusions are precipitates of myosin heavy chains found in the cytoplasm of neutrophils, eosinophils, monocytes, and basophils [3].

Most patients do not present with clinically significant bleeding, and treatment is not necessary. In the rare cases where severe hemorrhage occurs, platelet transfusion may be required [3]. Treatment with corticosteroids and splenectomy are ineffective. Medication that interferes with platelet function, such as aspirin, should be avoided [4]. Some patients may present with other clinical manifestations such as sensorineural hearing loss, cataracts, and renal failure.

CASE

Female patient, 23 years old, originally from Timor, seeks hematology consultation for follow-up of thrombocytopenia, known since 2007. No complaints and without evidence of hemorrhagic dyscrasia.

Analytically, the results showed: hemoglobin 13.3 g/dL, leukocytes 6200/µL (differential count without alterations) and platelets 32000/µL (by impedance method) and 89000/µL (by optical fluorescence method) [reference values 150 to 400000/µL]. The blood smear, stained with May-Grünwald--Giemsa stain, confirmed the presence of thrombocytopenia, with mostly giant platelets. (figures A, B and C) and basophilic inclusions in the cytoplasm of neutrophils and basophils, such as Döhle bodies (Figures D, E, and F). Renal and hepatic function normal. IgG antiplatelet antibodies negative (<120). Previous blood tests showed platelet count that fluctuated between 26 - 46000/µL, quantified by impedance method and 77 - 89000/µL, by optical fluorescence method or by estimated count in peripheral blood smear. The evaluation of platelet function (PFA-100), with the agonists Collagen/ADP and Collagen/Epinephrine, showed a slight alteration compatible with thrombocytopenia.

Given a thrombocytopenia with giant platelets and cytoplasmic inclusions in neutrophils and basophils, resembling Döhle bodies, the diagnostic hypothesis of Anomaly of *May-Hegglin*.

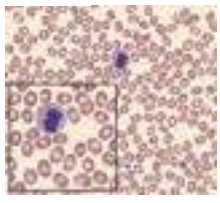

Figure A: Neutrophils with basophilic inclusions Döhle body type and giant platelets

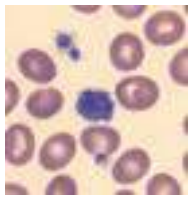

Figure B: Macrocytic platelets and giants

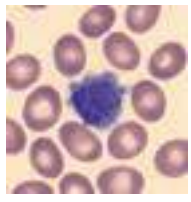

Figure C: Giant plaque

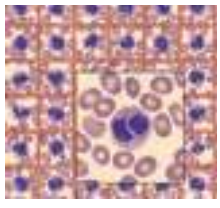

Figure D: Neutrophils with basophilic

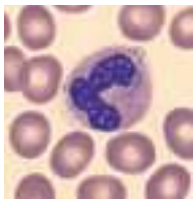

Figure E: Neutrophil with inclusions basophilic

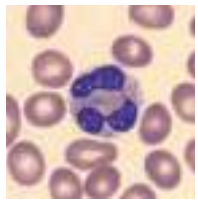

Figure F: Neutrophil with inclusions basophilic

DIFFERENTIAL DIAGNOSIS

The observation of cytoplasmic inclusions, such as Döhle bodies, in the presence of macrothrombocytopenia is a finding that helps to rule out other causes of hereditary macrothrombocytopenia, such as the syndrome of *Bernard--Soulier*, Gray platelet syndrome or disease of *von Willebrand* type IIB, or acquired macrothrombocytopenia, such as Immune Thrombocytopenic Purpura [4].

DISCUSSION

The case presented reflects the crucial role of the Hematology laboratory in the differential diagnosis of thrombocytopenia. Careful observation of the peripheral blood smear is essential to avoid incorrect diagnoses and unnecessary or inappropriate treatments.

In a confirmed case of thrombocytopenia, it is essential to perform a morphological evaluation of platelets under a microscope, paying particular attention to their cytological characteristics, such as size and the presence of granules. The identification of giant platelets in the smear should raise suspicion that the automated count may be underestimated, since these can be confused with erythrocytes and therefore excluded from the platelet count. In these cases, the use of more reliable complementary methods is recommended, such as fluorescent optical counting [5] or manual counting estimated in peripheral blood smears.

The authors emphasize the importance of considering the possibility of a diagnosis of thrombocytopenia and giant platelets when faced with this condition. *May-Hegglin* or another genetic alteration of MYH9. In these cases, careful research into basophilic cytoplasmic inclusions in neutrophils — type of bodies of *Döhle* — This can be crucial for diagnostic guidance.

POINTS TO HIGHLIGHT

- Automated platelet counts may be underestimated in the presence of giant platelets, and confirmation by complementary methods, namely manual estimation, is recommended.
- The presence of thrombocytopenia associated with giant platelets and basophilic cytoplasmic inclusions in granulocytes, of the type of blood bodies *Döhle* is strongly suggestive of the anomaly of *May-Hegglin*.
- The definitive diagnosis is established by identifying the mutation in the MYH9 gene.
- The anomaly of *May-Hegglin* It is characterized by a course Clinically, it is generally benign, and usually sufficient.

- Correctly recognizing the anomaly is crucial to avoid unnecessary or potentially harmful therapies.

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CASE N.º 3

Diagnosis of Hemophilia C in pregnant woman at 35 weeks

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ABSTRACT

We report a clinical case of a 40-year-old woman, 35 weeks pregnant, without a clinical history of spontaneous bleeding or bleeding during previous labours, who presented a prolonged activated partial thromboplastin time (aPTT) in routine tests during the third trimester. Through the mixing test, it was possible to verify the correction of the aPTT, which indicated a deficiency of a coagulation factor. Subsequently, the coagulation factor tests revealed a factor XI deficiency, confirming the diagnosis of haemophilia C.

INTRODUCTION

Hemophilia C is a rare genetic disorder caused by a deficiency of the clotting protein factor XI.

This disease affects 1 in 100,000 people, but is more frequent in Ashkenazi Jews and their descendants (prevalence of 8% in this community). This low prevalence is explained by its autosomal recessive genetic transmission pattern. Hemophilia C can be caused by a variety of genetic mutations, which justifies the great variability in the clinical picture. Unlike classic Hemophilia A and B, Hemophilia C affects men and women equally, since the mutated gene is located on chromosome 4.

The severity of symptoms of this disease does not correlate directly with the level of factor XI, which makes it difficult to predict the severity and frequency of bleeding episodes.

Hemorrhagic episodes can occur after physical trauma, particularly after surgeries involving the oral mucosa, nose, or urinary tract, but rarely spontaneously. Tooth extraction, tonsillectomy, and hysterectomy or prostatectomy are examples of surgeries that carry a higher risk of bleeding, and in this context, the disease may be the first manifestation. These patients have a high tendency to experience nosebleeds and hematomas, but rarely develop hemarthrosis or spontaneous intracranial hemorrhage.

CASE

A 40-year-old female patient, 35 weeks pregnant, with no history of spontaneous bleeding and/or bleeding during previous labor, undergoes routine third-trimester blood tests, which revealed a prolonged aPTT (63.5 sec). In this context, the attending physician contacts the Clinical Pathology Laboratory to understand this result. An admixture test was immediately performed, resulting in a corrected aPTT (25.3 sec), a result consistent with a diagnosis of a coagulation factor deficiency. At this stage, the clinical pathologist informs the attending physician of the result and initiates a sequential assay of intrinsic pathway coagulation factors, ultimately detecting a factor XI deficiency (9.5%). Given the diagnosis, the attending physician opts for induction of labor with the support of the immunohematology service. Screening for factor XI deficiency is performed on newborns who have not been confirmed [FXI=31% (reference values: 10-66%)].

DISCUSSION

Patients with Hemophilia C are generally asymptomatic; hemorrhagic manifestations occur after trauma. A clinical and family history of hemorrhagic episodes is very important for diagnosis. Performing hemorrhagic tests is also crucial. *screening* coagulation tests (PT, aPTT, and fibrinogen) are used to screen for this and other coagulation disorders in the preoperative context. Women, especially pregnant and postpartum women, constitute a risk group. Most are only diagnosed due to menorrhagia or postpartum hemorrhage.

Whenever a change is detected in the tests of *screening* For coagulation disorders, complementary tests should be performed for differential diagnosis. In this clinical case, the patient It presented with isolated aPTT prolongation. This prolongation isolated aPTT prolongation suggests a deficiency of an intrinsic pathway factor or the presence of an inhibitor. The differential diagnosis is based on performing a mixing test, which consists of determining the aPTT after mixing the patient's plasma with a pool of normal plasmas. Correction of the aPTT after mixing suggests a diagnosis of factor deficiency (by adding normal plasma to the patient's plasma, we correct the deficiency of the factor(s)). Failure to correct the aPTT after mixing suggests the presence of an inhibitor. In the reported case, since there was correction of the aPTT, sequential measurement of intrinsic pathway coagulation factors was performed (since it was an isolated aPTT prolongation), in order of frequency of prevalence of the deficits.

Hemophilia C, or factor XI deficiency, is a rare disease, but early diagnosis is crucial to prevent bleeding resulting from surgery or trauma, thus avoiding life-threatening situations. Diagnosis is difficult because, in most cases, patients do not bleed spontaneously, but it is essential before surgical interventions. The severity of the disease does not correlate with factor XI levels. Treatment or prevention of bleeding episodes can be achieved with factor XI concentrates, fresh frozen plasma, antifibrinolytic agents, or desmopressin, depending on the type.

POINTS TO HIGHLIGHT

- Hemophilia C (factor XI deficiency) is a rare, often asymptomatic coagulopathy whose clinical severity does not correlate with plasma levels of factor XI.
- It can be diagnosed incidentally during routine examinations, as happened in this case with an isolated prolongation of aPTT in a pregnant woman with no history of bleeding.
- The mixing test is essential in the differential diagnosis of prolonged aPTT, allowing distinction between factor deficiency and the presence of an inhibitor.
- In women, especially during pregnancy or childbirth, it is crucial to suspect and investigate coagulation disorders, given the increased risk of obstetric bleeding.
- Early diagnosis allows for the planning of appropriate preventive and therapeutic measures before surgical interventions or childbirth, reducing the risk of serious complications.

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CASE N.º 4

Analytical interferences: regarding a hidden monoclonal gammopathy

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ABSTRACT

Paraproteinaemias interfere with serum metabolite assays, producing spurious laboratory results. These events can be caused by analytical or pre-analytical interference due to the presence of monoclonal paraproteins in high concentrations. There are several examples described in the literature of interference in analytes as common as glucose, bilirubin, gamma-glutamyltransferase, urea, sodium, phosphorus, C-reactive protein, and ferritin. Indicators of haemolysis, icterus or lipaemia (HIL index) are common tools in current practice to detect interference in samples processed by large chemiluminescence autoanalysers. The real challenge is to identify mechanisms that can detect and avoid erroneous results or unnecessary tests. It is crucial to recognise the phenomena that produce an unexpected diagnosis.

The authors present two cases in which uric acid measurement revealed altered and unexpected results caused by the presence of paraproteinaemia. Exhaustive laboratory investigation of these results led to the identification of occult monoclonal gammopathy in both patients.

INTRODUCTION

There are several interference described in literature. [3,4] Despite current analyzers detecting changes in absorption during the course of a reaction, and thus the formation of turbidity due to M proteins, not validated mechanisms exist for analyzing samples in high-throughput laboratories. Paraprotein interference is relatively common in laboratory practice, and the presence of unexplained spurious laboratory results should prompt an investigation for an underlying paraprotein. On the other hand, it is expected that patients diagnosed with multiple myeloma may have anomalous results due to the presence of paraproteins in high concentrations.

CASE N°1

A 79-year-old man was seen by his family doctor for dizziness, headache, and fatigue. He had no reported history of cancer or gout and was not taking any medication other than a statin for elevated cholesterol.
Laboratory results revealed an extremely elevated uric acid level (16.3 mg/dl), outside the clinical context of mild normocytic and normochromic anemia (12.5 g/dl).
After several confirmatory tests, with various serum uric acid measurements (Table 1), we encountered the possibility of an analytical interference for this metabolite. We performed capillary electrophoresis of plasma proteins and found a peak in the gamma globulin region with an M component of 36.1 g/dl. By capillary electrophoresis with immunosubtraction, a monoclonal gammopathy of the IgM lambda type was identified.
Occult gammopathy with hyperviscosity syndrome was the true cause of the altered uricemia levels and explains the patient's symptoms of dizziness and headache.
No analytical interference was identified in other parameters. The patient was referred to a hematology clinic for further investigation.

CASE N°2

A 75-year-old man was examined by his family doctor during a routine annual check-up.
Uricemia assay revealed several errors and assay inability on the routine autoanalyzer (ADVIA® Chemistry XPT, Siemens). After two confirmatory stepsin other autoanalyzers with other methodologies (Dimen-In the analysis of sion® EXL, Siemens), we obtained normal levels of this metabolite (3.4/3.8 mg/dl) (Table 1). The possibility of analytical interference in this parameter was once again considered. Capillary electrophoresis of plasma proteins was performed, which revealed a peak in the gamma globulin region, with an M component of 30 g/dl, identified by capillary electrophoresis with immunoabsorptive assay as a monoclonal gammopathy of the IgG kappa type.
These findings were communicated and discussed with the family physician, and the patient was referred to a hematology clinic for further investigation.

RESULT

Both patients were referred to the hematology department for further investigation.

DISCUSSION

Monoclonal gammopathy is characterized by the presence of a monoclonal immunoglobulin or paraprotein in serum or urine and occurs in multiple myeloma, Waldenstrom's macroglobulinemia, plasmacytoma, amyloidosis, and monoclonal gammopathy of undetermined significance (MGUS). [1]
Paraproteins result from the proliferation of a single clone of plasma cells and are generally detected by the appearance of a single well-defined band in serum protein electrophoresis. The most common paraprotein is IgG (approximately 70%), with IgM and IgA being less common (approximately 17% and 11%, respectively). [2]
The presence of a large amount of monoclonal protein in serum can lead to a number of unusual laboratory results. One mechanism can be considered pre-analytical, related to the interaction of the paraprotein with a range of analytes, including cations, anions, enzymes, hormones, or lipids. The other is analytical interference resulting from precipitation or increased viscosity, interfering with optical readings.
There are several examples, reported in the literature, of the interference of gammopathy in analytes such as glucose, bilirubin, gamma-glutamyltransferase, urea, sodium, and phosphate levels . C-reactive protein, HDL, and ferritin. [3] Additionally, the Interferences caused by paraproteins are relatively common and appear to be dependent on the methodology used and their serum concentration. Song, Yang et al. [5] followed some patients, finding that as paraprotein concentrations tend to normalize after treatmenttoo, the interferences also disappear. The progression of Disease and

TABLE 1. SUMMARY OF LABORATORY RESEARCH AND RESPECTIVE METHODOLOGIES

ANALLYZER	METHODOLOGY	Uric acid dosages	
		Patient 1	Patient 2
ADVIA® XPT Chemistry, Siemens	Enzymatic reaction Uricase Fossati	< Conc. Range, less than 0: no result	< Conc. Range, less than 0: no result
Dimension® EXL, Siemens	Enzymatic reaction modified version of Uricase	16,3 mg/dl	3.8 mg/dl
		15,3 mg/dl	3,4 mg/dl
Arquitect® Abbot	Enzymatic reaction modified from Uricase Trivedi and Kabasakalian	8,8 mg/dl	-

increased concentrations of monoclonal protein cause increased interference.
Unexpected laboratory results, such as those presented in the cases presented here, can be confusing or misleading. They may trigger additional laboratory tests, further diagnostic tests, or even an incorrect treatment plan.

POINTS TO HIGHLIGHT

- Paraproteins are a common cause of unexpected laboratory results.
- Laboratories must quickly identify the results of these parameters and ensure that clinicians are aware of the potential for analytical interferences in laboratory tests.
- On the other hand, it is of utmost importance to recognize that careful laboratory investigation of these results may involve laborious techniques such as capillary electrophoresis, immunoabsorptive electrophoresis, or agarose gel immunofixation, revealing an unexpected diagnosis.

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CASE N.º 5

Macro TSH in pregnancy

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ABSTRACT

Recent technological advances have led to significant improvements in the accuracy of laboratory tests used for serum thyroid hormone assays.

The presence of analytical interference should be considered in case of discrepancies between TSH, free T4 and free T3 results in the absence of clinical signs or symptoms of hypothyroidism. Heterophile antibodies or the presence of a macro-TSH form resulting from an IgG immune complex bound to anti-TSH autoantibodies can lead to an erroneous clinical interpretation of the results. During pregnancy, the thyroid gland is under considerable demand, requiring significant maternal physiological adaptations, which are particularly important in case of thyroid disease history. The presence of anti-thyroid autoantibodies should be excluded in these cases, as Ig G crosses the foeto-placental barrier, inducing hypothyroidism in the foetus. We conducted a literature review and found a small number of cases documented in the last 10 years. The prevalence of elevated TSH due to the presence of macro-TSH was 0.6%. We used the polyethylene glycol (PEG) precipitation test as a method for separating the TSH monomer from the immunocomplex. We report the case of a 33-year-old pregnant woman who, in the second trimester laboratory evaluation, revealed a slight increase in TSH, 5,850 mIU/L, measured by a 4th generation chemiluminescence immunoassay (Advia Centaur XP™, Siemens), with normal free T4 and T3 fractions, without signs or symptoms of hypothyroidism. After the PEG precipitation method, the recovery rate was 56%, suggesting the presence of analytical interference in TSH measurement due to the presence of immune complexes.

INTRODUCTION

Over the past four decades, technological advances have allowed for an increase in the sensitivity and specificity of laboratory tests used for serum measurements of thyroid function hormones.

The use of third- and fourth-generation assays for thyroid-stimulating hormone, TSH or thyrotropin, as well as the established log-linear relationship with free thyroxine (T4), have established TSH as the most sensitive marker for testing thyroid function. Discrepancies between TSH results and free T4 and T3 fractions, without symptoms or clinical signs of hypothyroidism, should lead the laboratory to suspect an analytical interference. The presence of heterophilic antibodies or anti-TSH antibodies forms macromolecular immune complexes, resulting in a form of macro-TSH without biological activity, but which produces altered TSH levels [1,2,7,9]. These analyte complexes-Antibody-related symptoms are a well-known cause of clinical misinterpretation of laboratory results. This phenomenon is also cited as a cause of macroprolactinemia [3] or even interference in serum enzyme assays such as creatine kinase (CK) or amylase, requiring a high degree of suspicion in identifying these laboratory findings.

This phenomenon is especially important during pregnancy, since IgG immune complexes such as macro-TSH or antithyroid antibodies cross the fetoplacental barrier, inducing fetal hypothyroidism [6,8].

We conducted a literature review of the last 10 years and found a small number of documented cases. The prevalence of elevated TSH due to macro-TSH was 0.6% in the general population [2,9] and there are no data in the literature on prevalence during pregnancy.

CASE

We examined a healthy 33-year-old woman, 20 weeks pregnant, with no history of thyroid disease, presenting with a slight elevation of TSH, 5,850 mIU/L (0.2-3.0), normal free T3 and free T4, 3.51 pg/mL (2.30-4.20) and 0.99 ng/dL (0.80-1.75), respectively, without signs or symptoms of hypothyroidism. Anti-thyroid antibodies, anti-thyroglobulin, anti-peroxidase, and anti-TSH receptor antibodies (TRABs), were negative. After the PEG precipitation test, the TSH concentration was measured again, and the presence of macro-TSH in the total TSH concentration was calculated. The recovery rate was 56%, with a corrected TSH value of 2,100 mIU/L, suggesting the presence of the TSH monomer and analytical interference in TSH measurement due to the presence of immune complexes.

INVESTIGATION

We used the fourth-generation TSH chemiluminescence immunoassay on the Siemens Advia Centaur XP™ autoanalyzer to measure TSH, free thyroxine (FT4), and free triiodothyronine (FT3).

For anti-thyroid, anti-thyroglobulin, and anti-peroxidase antibodies, we used the Immulite 2000™ two-site chemiluminescent immunometric assay, DPC.

Anti-TSh receptor antibodies (TRAB) were analyzed by radioimmunoassay (RIA).

As a method for separating the immune complex, we used the polyethylene glycol (PEG) precipitation test, PEG 6000 (Wako Junyaku kogyo kk ™, Tokyo, Japan).

Table 1 summarizes the laboratory investigations carried out.

DISCUSSION

Interference from the assay in TRH measurement is relatively common. Some authors report that in approximately 0.5 to 5% of samples tested for thyroid function, the TSH measurement is analytically suspected of being altered by an analytical interference [2]. Spurious TSH elevation should be considered and investigated when incongruent with the clinical presentation, particularly in elevations greater than 10 mIU/L [2]. However, small changes in reference values should also be investigated with additional laboratory methods outside the usual routine, otherwise unnecessary clinical investigations and therapeutic decisions may be made.

The generally accepted reference value for TRH in circulating blood is 0,350-5,500 mIU/L [6]. TSH secretion is under the control of T3 level and thyrotropin-releasing hormone (TRH). In some situations, the reciprocal relationship between serum TSH and free fraction concentrations is disrupted. Interference in the assay, such as the presence of anti-TSH antibodies, is one such situation [2,7,8,9,10].

Thyroid disease in pregnancy is a common clinical entity, [1,3,5,6,7,9,10] 20% of pregnant women have positive antithyroid antibodies, 2 to 3% have undiagnosed hypothyroidism and 0.3 to 0.5% have hyperthyroidism [3].

The interpretation of TSH values during pregnancy should be done according to the values adjusted for gestational age as suggested by Stagnaro-Green et al [5] mentioned as evidence III b in the General Directorate of Health Standard in 2011: in the 1st quarter: 0.1 – 2.5 mU/L; in the 2nd quarter: 0.2 – 3.0 mU/L and in the 3rd trimester: 0.3 – 3.0 mU/L. [6]

The presence of macro-TSH appears to affect most commercially available TSH assays. Causes of analytical interference

TABLE 1. Summary of results

Laboratory investigations	Results	Conclusions
1. Whithout treatment		
TSH, Advia™ Centaur-Siemens	5.850 mUI/L (0,2-3.0)	Elevated TSH with euthyroidism
F T3, Advia™ Centaur-Siemens	3,51 pg/mL (2,30-4,20)	clinical and laboratory.
F T4, Advia™ Centaur-Siemens	0,99 ng/ dL (0,80-1,75)	Suggestive of analytical interference
2. Precipitation with PEG and reanalysis of the supernatant		
TSH, Advia™ Centaur-Siemens	2,10 mUI/L (0,2-3.0)	Analytical interference by
% TSH recovery	56%	macromolecules

reported in the literature include the presence of sample of heterophilic antibodies, human antibodies anti-mouse or anti-rabbit antibodies and the presence of a positive rheumatoid factor [2,7,8,9,10]. The common mechanism for positive interference in this assay is the cross-linking of capture and signal antibodies from the sandwich immunoassay, falsely generating a detection signal with an increased TSH result [2,8]. In parallel, because it is a macromolecule, the renal clearance of macro-TSH is markedly reduced, with an increase in its serum concentration and an elevation in TSH levels. The biological activity attributed to the presence of macro-TSH is reduced [2,7,8,9,10], so patients remain clinically and laboratorially euthyroid.

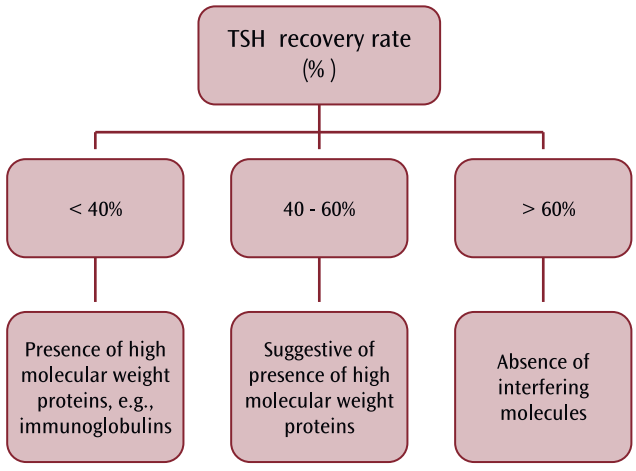
All clinical diagnostic laboratories should perform additional investigative tests when altered TSH results are inconsistent with the patient's clinical condition and/or with normal free thyroxine values. This issue is particularly important in the laboratory monitoring of pregnant women with thyroid disease and in neonatal screening, but should be applied to any situation.

One of the methods of choice is the polyethylene glycol (PEG) precipitation test. [2,4,7,8,9,10] PEG acts as a chelating agent, adsorbing any heterophilic antibodies and other immunoglobulins present in these samples, allowing the discrimination of interfering substances from true cases of elevated TSH.

In our laboratory, we performed the PEG precipitation test, according to the procedure described by Sakai et al [8], dissolving 2.5 g of PEG 6000 (Wako Junyaku kogyo Kk, Tokyo, Japan) in 10 mL of distilled water. Equal volumes of a 25% PEG solution and patient serum are mixed and centrifuged at 3000 g for 5 min. The supernatant is separated and the TSH level is measured using the Advia Centaur XP™, Siemens. The TSH recovery rate is calculated using the following formula [8]:

TSH recovery rate (%) = 2 x TSH (after absorption) / TSH (before absorption) x100.

The interpretation of the results is done according to the following your algorithm [8]:



In this case, the recovery rate was 56%, with a value the corrected TSH level was 2,100 mIU/L, normal for gestational age, suggesting the presence of the TSH monomer and analytical interference in TSH measurement due to the presence of immune complexes. Early recognition of this interference in the TSH assay can prevent diagnostic errors and unnecessary investigation and/or treatments.

POINTS TO HIGHLIGHT

- Macro-TSH is an underdiagnosed analytical interference.
- The laboratory techniques described can diagnose this rare entity, which causes isolated TSH elevation in clinically euthyroid individuals.
- Thyroid disease in pregnancy is a common clinical entity, and patients with macro-TSH should be monitored, especially those with positive anti-thyroid antibodies.
- Close dialogue between the clinician and the laboratory is crucial in managing these cases, allowing for the detection of macro-TSH, preventing the initiation of hormone replacement therapy for a possible case of subclinical hypothyroidism.

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CASE N.º 6

Ocular myiasis caused by Oestrus ovis in Portugal: a clinical case

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ABSTRACT

Human myiasis is more prevalent in rural areas [3,11] due to the increased exposure to host animals and sanitary conditions favourable to the life cycle of the causative diptera. However, cases have also been reported in urban areas [7,8]. The larvae can invade or infest different human tissues and the condition is classified as ophthalmomyiasis or ocular myiasis when they invade the eye or ocular region, causing significant damage. [4,6,12]

The authors report the case of a 26-year-old man, on holidays in southern Portugal (Algarve) who, while walking in a rural area, 'felt something hitting his eye', followed by a foreign body sensation and difficulty in opening his eye. In the hospital emergency department, twelve larvae were observed, associated with mild inflammation of the conjunctiva. One of the larvae was morphologically identified as belonging to the species *Oestrus ovis* (*Oestridae* family) and classified as external ophthalmomyiasis. [3,4] Molecular confirmation of the insect larva sample was attempted by amplifying the mitochondrial DNA of cytochrome oxidase subunit I (COI) using LCO1490 and HCO2198 *primers* [1]. However, the attempt was unsuccessful, most likely due to the quality and small quantity of material received by the laboratory.

Early diagnosis allowed for rapid intervention with removal of the larvae to prevent residual lesions, reducing complications and destruction of ocular structures. To our knowledge, this is the first reported case of ocular myiasis in Portugal caused by *Oestrus ovis*. The case serves as a warning for the diagnosis of these infestations in southern European countries, especially in rural areas [13], as well as for the importance of early diagnosis.

INTRODUCTION

Myiasis is a parasitic disease of vertebrate tissues caused by the larvae of dipteran ectoparasites (order Diptera).[2] Several species of flies cause traumatic or wound myiasis, including flies from the families *Calliphoridae*, *Musci-dae*, *Oestridae*, *Phoridae* and *Sarcophagidae*. The occurrence and prevalence of myiasis are associated with environmental factors, namely the abundance and distribution of the fly population and the susceptibility of vertebrate hosts.

humana myiasis generally occurs in rural areas where people live in direct contact with animals, but it can also occur in urban areas. The larvae of the species mentioned above affect different human tissues. Ophthalmomyiasis or ocular myiasis represents less than 5% of all myiasis cases. This pathology can be divided into external forms (99.62% of cases described in Mediterranean countries) and internal forms (approximately 0.38%).

Ophthalmyiasis is typically seen in farmers, herders, and travelers in rural areas. *Oestrus ovis* It is the most common cause of external ophthalmyiasis.

Given that this condition is very uncommon in Portugal, doctors may not consider this diagnosis.

In the case described, identification of the larvae was only possible based on clinical-epidemiological data and the morphology of the cephalopharyngeal skeleton of the L1 larva.

CASE

A 26-year-old man, residing in the USA and on vacation in southern Portugal (Algarve), suffered a sudden trauma to his right eye during a walk in a rural area, followed by a sensation of a foreign body and difficulty opening his eye. On the same day, the patient went to the emergency room of the Lagos hospital complaining of eye pain.

Physical examination revealed the presence of twelve live larvae in the cornea, along with mild conjunctival inflammation. The larvae were removed with a swab and placed in a sterile, dry tube for microbiological analysis. The eye was washed and topical antibiotics were applied. After 24 hours with the eye protected, the patient was referred for an ophthalmological consultation where it was confirmed that all larvae had been removed. There was no evidence of tissue damage, and the patient was discharged with medication for topical antibiotics and corticosteroids.

Molecular identification of the larva was attempted using cytochrome oxidase (COI) subunit I. Mitochondrial DNA was then amplified by molecular biology (PCR) using the following methods: *primers* LCO1490 and HCO2198. The larval sample received in the laboratory was homogenized with 200 µl of lysis buffer using a *Mixer Mill MM400*® (Retsch GmbH, Haan, Germany) with a 3 mm steel ball. Nucleic acids were extracted from the resulting homogenate (NUCLISENS® *easyMAG*, Biomérieux). Total nucleic acid extraction was performed on the automated NUCLI-SENS® platform *easyMAG* (Biomérieux) and eluted in 60 µl of buffer solution.

For PCR amplification, 10 µl of DNA and 10 pmol of each first, added to the reagent *FastStart PCR Master*, with a final reaction volume of 25 µl (Roche, Basel, Switzerland). The PCR conditions were as follows: denaturation at 95 °C for 3 minutes, 40 cycles of 94 °C for 20 s, 50 °C for 20 s and 72 °C for 30 s, with a final extension at 72 °C for 5 minutes.

Since the amplification resulted in a weak band (observed a second round of amplification was performed using the same PCR protocol (using 1.5% agarose gel electrophoresis - TAE 1x). The amplicons obtained were purified using the *kit JETquick PCR product purification spin* (GENOMED GmbH, Löhne, Germany) and sequenced with the ABI Prism 3130® genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Molecular identification was not possible due to the low concentration of DNA obtained from the extraction of a single larva. The sequence results were not of sufficient quality to obtain the COI sequence.

A larval trace was studied under a stereomicroscope using identification keys for third-stage larvae of myiasis-causing flies. The posterior spiracular plates were not present in the larval trace; therefore, identification was based on the cephalopharyngeal skeleton. Both structures are commonly used in morphological identification.

The larva was morphologically identified as a larva of *Oestrus ovis* (family *Oestridae*), and the diagnosis of ophthalmomyiasis was confirmed (Figure 1).

RESULTS AND MONITORING

The patient returned to the US, having reported no long-term consequences.

DISCUSSION

Human myiasis has a worldwide distribution, with a greater number of species and greater abundance in tropical and subtropical regions of countries with low socioeconomic levels. The main risk factors are poor hygiene and the presence of suppurative lesions (an important factor for egg deposition). Most cases are diagnosed accidentally, and the true impact of myiasis is unknown due to underreporting and the lack of identification of the causative agent.

Myiasis can be classified into: hematophagous myiasis (blood-feeding), cutaneous myiasis (wound, furuncular, and migratory forms), cavitary myiasis (e.g., ocular, oral, nasopharyngeal), urogenital and intestinal myiasis. Pseudomyiasis is characterized by the accidental ingestion of larvae. The most frequent form is cavitary myiasis, where the infestation receives the name of the affected organ, as in the case of ophthalmomyiasis.

Ocular myiasis occurs more frequently in the spring and summer, and is most commonly caused by Oestrus ovis, but other species have already been described, such as *Gasterophilus sp.*, *Wohlfahrtia magnifica*, *Chrysomya bezziana*, *Wordylobia anthropophaga* and *Dermatobia hominis*, among others.

Oestrus ovis is a widely distributed fly species known for its parasitism, which can have a major impact on veterinary health, [9,10] with significant losses in animal production. It mainly affects sheep, deer and goats, but can occasionally be found in cattle, horses, dogs and humans.

The life cycle of Oestrus ovis It begins with the laying of eggs, which are fertilized and hatch inside the female's body, forming larvae of about 1 mm. The female deposits some larvae, in a small drop of mucus, directly into the nostril of the host animal. The first-stage larvae cross the nasal mucosa, enter the sinuses, and develop into the second larval stage. The second-stage larvae grow to about 20 mm (4/5 of an inch) in length. When fully developed, they descend through the nasal canal and fall to the ground, where they burrow for pupation. The life cycle depends on ambient temperature—it can last from 25 to 35 days in warm climates, but up to 10 months in cold climates. The pupa takes 3 to 9 weeks to mature, after which the adult fly emerges.

Ophthalmomyiasis is considered external [4,6,7,13] (or superficial) when the infestation occurs in the superficial tissues of the eye, and internal when there is intraocular invasion. In the external form, conjunctival myiasis is the most frequent. *Oestrus ovis* It is the principal agent of ophthalmomyiasis externa, and most cases are observed in rural areas of the north and south with cooler climates. Symptoms are acute and related to movement and the inflammatory response induced by the larva. The number of larvae observed varies (between 5 and 18).

Internal ophthalmomyiasis can be a complication of the external form, with fewer larvae involved (usually one) and more severe symptoms: red eye, eye pain, vision loss, and scotomas. Other fly species associated with internal ophthalmomyiasis are *Dermatobia hominis* and *Hypoderma spp*.

In the case presented, the possibility of external ocular myiasis was considered based on the epidemiological context and the acute and characteristic onset of symptoms, such as a foreign body sensation and eye pain. Macroscopic visualization of twelve larvae in the affected eye and subsequent morphological identification of the species were performed. *Oestrus ovis* a reference laboratory confirmed the diagnosis. Molecular identification was attempted after morphological identification, but the amount of biological material available was small and of low quality, making DNA extraction, PCR amplification, and consequently, COI gene sequencing unfeasible.

Complications such as corneal ulcers, retinal detachment, orbital cellulitis, and vision loss are rare and did not occur in this patient.

Treatment for external ocular myiasis consists of applying a local anesthetic (which also reduces larval activity), followed by their mechanical removal. Topical antihistamines and antibiotics may be prescribed if necessary.

Although some cases have been described in several European countries, in Portugal there are only two previously published cases of myiasis. This is the first described case of ocular myiasis with species identification in Portugal, and serves as a warning of the possibility of these infestations in southern European countries [13], especially in rural areas. It also highlights the importance of early diagnosis and treatment, with rapid removal of larvae, in order to avoid residual lesions.

POINTS TO HIGHLIGHT

- This case serves as a reminder of a diagnosis that is uncommon in southern European countries.
- Early removal of the larva is crucial to reduce the inflammatory process and prevent eye damage.
- Diagnosing cavitary myiasis is challenging, especiallyte for clinicians who are not familiar with this illness.

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CASE N.º 7

Individuals with balanced translocations and recurrent miscarriages - a case study

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ABSTRACT

Balanced structural chromosomal abnormalities, particularly reciprocal translocations, are one of the genetic causes most frequently associated with recurrent pregnancy loss. We present the case of a 38-year-old woman with two spontaneous abortions and a suggestive family history, namely multiple pregnancy losses in her mother and sister, who is a carrier of a balanced familial translocation. The presence of balanced translocations can lead to gametes with chromosomal imbalances, resulting in infertility, miscarriages or offspring with malformations. Identifying this type of disorder not only allows the cause of reproductive losses to be established, but also enables appropriate genetic counselling and consideration of reproductive strategies such as pre-implantation genetic diagnosis or prenatal diagnosis in future pregnancies.

INTRODUCTION

Several studies on pregnancy disorders indicate that spontaneous abortion is one of the frequent complications, totaling about 10-15% of clinically recognized pregnancies. However, recurrent miscarriage, currently defined by the ESHRE (European Society of Human Reproduction and Embryology) as the occurrence of 2 or more pregnancy losses, is not as frequent and occurs only in approximately 1-2% of women [1]. Although they are known some of the risk factors for pregnancy loss include...

Although there are conditions such as Antiphospholipid Syndrome, endocrine disorders, chromosomal abnormalities, some uterine anomalies, among others, the vast majority of cases remain without a known etiology.

Chromosomal abnormalities are the most frequent genetic cause responsible for recurrent pregnancy losses.[2]

In approximately 2-8% of couples with recurrent pregnancy loss, at least one of them carries a chromosomal abnormality, the most frequent being balanced structural chromosomal abnormalities. In young couples with a family history of recurrent miscarriages, the probability increases that one of the partners may carry a balanced chromosomal abnormality. Balanced structural chromosomal abnormalities include reciprocal translocations (61%), Robertsonian translocations (16%), pericentric inversions (8%), and paracentric inversions (8%) [3,4].

Reciprocal translocation, the most frequent of structural chromosomal alterations, is an anomaly involving at least two breaks in two different chromosomes and the exchange of the respective segments between them. This change can be balanced or unbalanced, occur de novo, or be inherited. Generally, a carrier of a balanced translocation does not present phenotypic anomalies; however, they have an increased risk, compared to a non-carrier, of presenting reproductive alterations such as infertility, recurrent miscarriages, and offspring with malformations and/or mental retardation.

CASE

We present the clinical case of a healthy 38-year-old woman who was asked to undergo cytogenetic testing due to a personal history of two spontaneous abortions. The first occurred in 2008, at approximately 10 weeks of gestation, and the second occurred in 2019, at 7 weeks of gestation.

She has a 4-year-old son who, apparently, has appropriate psychomotor development for his age.

In the family history, it is worth highlighting that in the obstetric history of her mother, currently 70 years old, there is reference to 4 spontaneous abortions, which always occurred between 6 and 8 weeks of gestation and in the interval of other successful pregnancies [3]. It should also be noted that her sister, 48 years old, in the interval of two normal pregnancies, also had 2 spontaneous abortions, which occurred at 6 and 7 weeks of gestation respectively.

METHOD

Karyotyping from peripheral blood was performed according to standard protocol; chromosomes were stained using G-banding; 20 metaphases were analyzed.

Through the Cytovision automated karyotyping system (Leica) and the alteration identified in the cytogenetic study was reported in accordance with the International System for Human Cytogenomic Nomenclature (2020).

RESULT

The cytogenetic study performed on the couple revealed in the woman the existence of a reciprocal and apparently balanced translocation between the long arm of one of chromosomes 9 and the long arm of one of chromosomes 16 with breakpoints at 9q34.1 and 16q22 – (Figure 1).

Subsequently, cytogenetic studies were performed on his parents, confirming that the (9;16) translocation was inherited from his father – (Figure 2). The sister of the index case, who had a history of recurrent miscarriages, also showed that she was a carrier of the same familial translocation – (Figure 3).

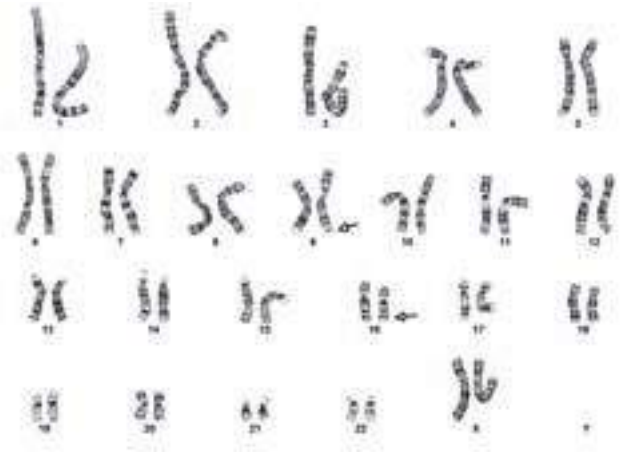

Figure 1. Karyotype: 46,XX,t(9;16)(q34.1;q22).

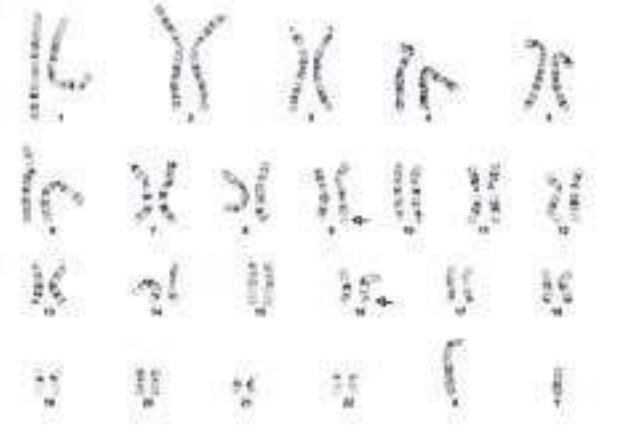

Figure 1. Karyotype: 46,XX,t(9;16)(q34.1;q22).

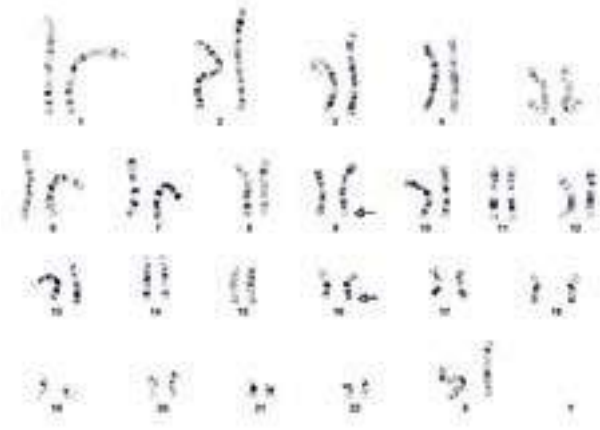

Figure 3. Karyotype: 46,XX,t(9;16)(q34.1;q22).

DISCUSSION

Pregnancy loss is always a situation that carries a strong physical and emotional impact for any couple, therefore, identifying its causes is extremely important for adequate support of these families. We present the case of a family carrying a balanced reciprocal translocation between chromosome 9 and chromosome 16, with a history of recurrent miscarriages in at least two generations.

Balanced chromosomal translocations are the most frequent genetic cause in couples who experience recurrent pregnancy loss.

Individuals with reciprocal translocations have an increased risk of having offspring with balanced chromosomal abnormalities, therefore identical to those of their parents, or unbalanced abnormalities, resulting from the presence of monosomies and/or partial trisomies of the chromosomes involved.

Some of the unbalanced changes will not be compatible with a full-term pregnancy, and an increased incidence of spontaneous abortions and live births with specific syndromes results from anomalies is also expected multiple congenital and/or developmentally abnormalities to psychomotor.

Some of the unbalanced changes will not be compatible with a full-term pregnancy, and an increased incidence of spontaneous abortions and live births with specific syndromes resulting from multiple congenital anomalies and/or psychomotor developmental disorders is also expected.

Identifying the presence of chromosomal abnormalities in couples with recurrent miscarriages allows us to understand the cause of these miscarriages, assess the risk of recurrence in future pregnancies, and offer the possibility of prenatal diagnosis of chromosomal abnormalities in those same pregnancies.

In this context, whenever a chromosomal abnormality is diagnosed in one of the members of the couple, a Genetic Counseling consultation will be recommended, with an indication for Prenatal Diagnosis and, in particular cases, the proposal of Preimplantation Genetic Diagnosis in future pregnancies.

Cytogenetic study of the parents is also indicated in order to establish the inheritance of the chromosomal alteration found, so that any relatives at risk can be identified [5].

POINTS TO HIGHLIGHT

- Balanced reciprocal translocations are a frequent and silent genetic cause of recurrent miscarriage—the carrier is usually phenotypically normal but has an increased reproductive risk.
- The need for genetic counseling after the identification of a balanced translocation, to assess future reproductive risk and inform about reproductive options such as preimplantation genetic diagnosis (PGD) or prenatal diagnosis (PND).
- This study shows the importance of including family history in cases of recurrent miscarriage—a detail that is often overlooked.

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CASE N.º 8

Newborn with tetrasomy 18p after a low-risk NIPT result - the importance of good pre-test counseling

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ABSTRACT

Non-invasive prenatal testing has increasingly taken on a central role in foetal aneuploidy screening, with high sensitivity for trisomies 13, 18, and 21. However, its growing use as an alternative to invasive techniques raises concerns about the understanding of its limitations. We present the case of a newborn with a dysmorphic phenotype and a postnatal diagnosis of tetrasomy 18p, despite a low-risk prenatal NIPT result. Tetrasomy 18p is a rare condition associated with advanced maternal age, manifested by craniofacial dysmorphia, neurodevelopmental delay, and other abnormalities. This case reinforces the importance of clear and comprehensive genetic counselling prior to testing, emphasising that NIPT, although valuable, does not replace cytogenetic diagnosis nor does it rule out rare anomalies.

INTRODUCTION

Since 2012, non-invasive prenatal testing (NIPT- *Non-invasive Prenatal Testing*) is used worldwide as a first-line test or applied contingently in the screening of trisomies 13, 18, and 21. The test is based on highly complex molecular methods that analyze cell-free placental DNA from maternal blood plasma. Recent studies suggest that NIPT may replace conventional combined screening for Patau, Edwards, and Down syndromes performed on all pregnant women from 10 weeks onwards. The various commercially available tests use different methodologies, all of which are highly complex and may represent a challenge for healthcare professionals regarding genetic counseling for couples who choose to undergo NIPT, given that this is a continuously and rapidly evolving field [1,2,3].

This article describes the case of a 39-year-old woman who opted for a non-invasive prenatal test at 15 weeks of gestation as a screening test for major aneuploidies. This test was performed as an alternative to amniocentesis due to the advanced maternal age and resulted in a *low risk* for major aneuploidies. At 37 weeks and 6 days of gestation, a male baby was born by elective cesarean section due to a restriction the of fetal growth.

METHODS

The genetic study performed on the newborn after birth was an array (aCGH) and this was complemented by conventional cytogenetics (karyotype). Both tests were performed using a peripheral blood sample.

a) CGH:

The sample was hybridized with a commercial reference sample of male human DNA (Promega biotech). For the detection of copy number variations (NVCFor studies related to intellectual disability and/or dysmorphic syndromes, the Agilent array-CGH (manufactured by Agilent Tech) was used. Data analysis was performed using the hg19 reference genome, and statistical analysis was performed using ADM-2 software. The aCGH analysis defines 5 as the minimum number of consecutive probes to consider the results valid. NVCs detected. The syndromic regions included in this format are covered by an estimated average resolution of approximately 100 kb and 350 bp for the rest of the genome.

b) Karyotype:

For cytogenetic characterization of the result obtained by arrayCGH, two independent cultures of phytohemagglutinin--stimulated lymphocytes were performed from a peripheral blood sample. The cultures were collected after 72 hours of incubation at 37°C with *colcemid* Thirty cells were karyotyped using the Cytovision automated karyotyping system (Leica). The chromosomes were previously stained with GTG bands, and cytogenetic analysis was performed according to the parameters provided by the International System of Nomenclature Cytogenetics Human (ISCN 2016).

RESULT

The *NIPT* a test performed at 15 weeks of gestation revealed a low risk trisomy of chromosomes 13, 18, and 21. After birth parto and in the presence of a dysmorphic phenotype in the newborn, including due to the peculiar facial features, hypospadias, low-set ears, hypertonia of the limbs, and high-arched palate, an aCGH was requested.

aCGH analysis detected a triplication of the entire short arm of chromosome 18 (Figure 1).

Cytogenetic analysis revealed the presence of a supernumerary marker metacentric chromosome in all cells analyzed, consisting of two short arms of chromosome 18 (47,XY,+mar) (Figure 2), confirming the presence of an 18p isochromosome.

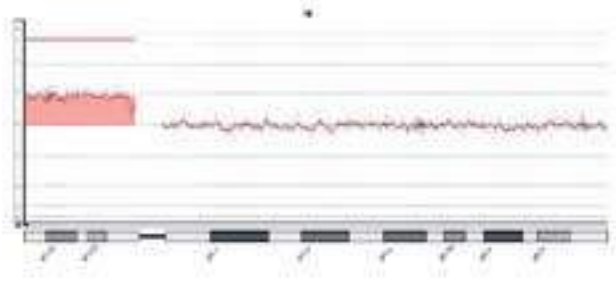

Figure 1. Anormal array CGH profile (Agilent 60K) - triplication of the entire

Figure 2.
chromosome 18s (left) and the supernumerary isochromosome 18p (right).

The parents underwent cytogenetic study in order to determine the heritability of isochromosome 18p and to accurately assess the risk of recurrence in future pregnancies. Both had normal karyotypes.

CONCLUSION

Isochromosomes are supernumerary chromosomes, consisting of two copies of the same chromosome arm, each arm forming a mirror image of the other arm, resulting in a tetrasomy of the arm involved.

Isochromosome 18p results in tetrasomy 18p (OMIM# 614290) and was first described in 1963 by Froland. It is a rare chromosomal abnormality with few described non-mosaic cases, with a mean survival beyond two years of age. It occurs in 1 in 140,000 live births, affecting females and males equally.

Most described cases of tetrasomy 18p are detected after birth and have an incidence of cases again strongly associated with advanced maternal age. Scientific studies suggest that isochromosome 18p occurs due to nondisjunction in meiosis II, frequently observed in women of advanced age [4].

The main characteristics of tetrasomy 18p are: low birth weight, hypotonia, moderate to severe cognitive impairment, marked language delay, little or no self-feeding ability. They also present with microcephaly, dolichocephaly, and craniofacial abnormalities such as facial features oval in shape, low-set ears, strabismus, small nose, long philtrum, central upper lip overlapping the lower lip, small mouth and

pronounced palatal arch [5,6].

The incorporation of NIPT into obstetric practice has had a significant impact. NIPT allows for higher sensitivities and specificities in prenatal screening for aneuploidies of chromosomes 13, 18, and 21, with lower false-positive rates compared to maternal serum markers. Additionally, NIPT allows for earlier results, even in the first trimester, and results in a decrease in invasive procedures associated with a risk of pregnancy loss afterward. While it is clear that this new screening option can offer significant benefits to women concerned about the risk of having a baby with a common aneuploidy, appropriate genetic counseling before testing is essential to ensure that expectant mothers are informed of the limitations of NIPT.

Pregnant women who choose to undergo NIPT need information about the advantages and limitations this prenatal screening method as the fact that this the test may not detect partial alterations of chromosomes 13, 18 and 21 (as in this case) and a low-risk result does not guarantee a pregnancy without alterations. It is necessary to consider that women undergoing the test and obtaining a low-risk result may have a false sense of security. Thus, it is necessary to provide adequate genetic counseling before the test is performed, explaining in detail its advantages and disadvantages, as well as its limitations compared to other methods, also ensuring good obstetric follow-up [7].

POINTS TO HIGHLIGHT

- Pre-test counseling is essential for a proper understanding of the limitations and advantages of taking the test
- Genetic, cytogenetic, or molecular diagnosis remains essential in cases with ultrasound abnormalities, even after "negative" tests.
- This case illustrates the risk of a false sense of security. provided by a low-risk NIPT result.

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CASE N.º 9

LE cell research. Is this test still useful for cases not suspected of Systemic Lupus Erythematosus (SLE)?

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ABSTRACT

The authors describe a case of a female patient diagnosed with systemic lupus erythematosus (SLE) with an exuberant clinical presentation in the context of fever and mucocutaneous manifestations. Laboratory investigation revealed changes in bone marrow aspirate morphology consistent with the cells described in 1948 by Hargraves, which we call LE cells. Although at the time it was considered a diagnostic criterion, it is now considered a low sensitivity test and has been replaced by antinuclear antibody (ANA) detection by indirect immunofluorescence in Hep2 cells. In clinical cases where SLE is not suspected (namely, to clarify cytopenia), identifying LE cells can provide important guidance for diagnosis and should not be underestimated in clinical practice.

CASE

MOPR, female, 53 years old, with fair skin, presented with anorexia, marked asthenia, weight loss, odynophagia, and high fever of approximately one month's duration. Physical examination revealed palatal edema, oral mucosal ulcers, urticarial exanthematous lesions on the back and lower limbs, and small cervical and inguinal lymphadenopathy.

LABORATORY INVESTIGATION

Among the complementary diagnostic tests, the following stand out: normocytic and normochromic, hyporegenerative anemia (Hb – 8.6 g/dL, MCV – 88.5 fL, MCH – 29.1 pg, reticulocytes – 0.04 x 10^12/L), leukopenia with lymphocytopenia (WBC – 3.0 x 10^9/L, L – 0.47 x 10^9/L), platelets in normal numbers (230 x 10^9/L), elevated ESR (63 mm), elevated ferritin (1217 ng/mL; reference value 10 to 291 ng/mL).
Peripheral blood smears showed rouleaux erythrocytes, without other significant alterations. The immunological study reveals consumption of the C3 and C4 complement fractions, 48.25 mg/dl (90-180) and 7.20 mg/dl (20-40) respectively, with ds-DNA positive after confirmation by reference methodology (indirect immunofluorescence in Crithidia luciliae) (figure 1).
The search for antinuclear antibodies by indirect immunofluorescence (IFI) in Hep2 cells was positive at a high titer (640), revealing a homogeneous pattern and a dense fine granular cytoplasmic pattern (figure 2).
The study of specific autoantibodies using the immunoblot methodology reveals the presence of anti-histones, anti-nucleosomes, and anti-ribosomal P antibodies, all at high titers (Figure 3).
Assessment of renal function reveals a marked decrease in creatinine clearance 64 ml/min (88-128), as well as the presence of proteinuria, 0.26 g/24h (<0.15).

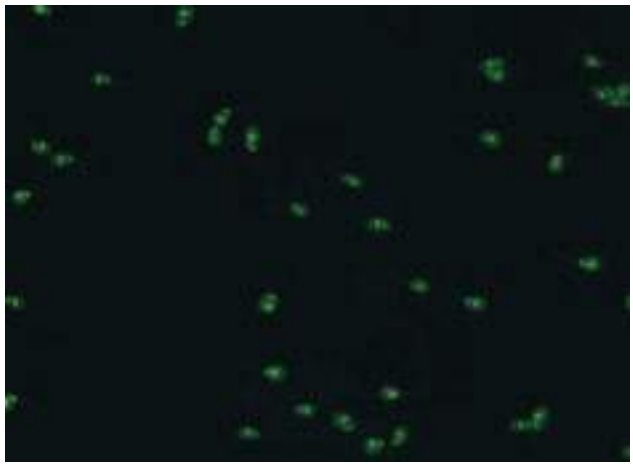


Figure 1. Indirect immunofluorescence in *Crithidia luciliae*.

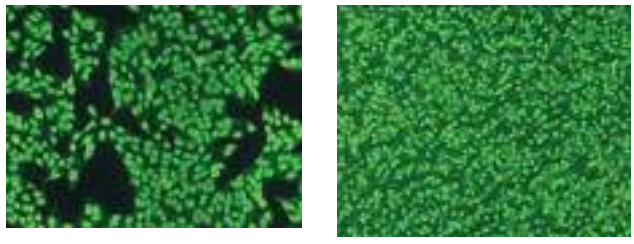


Figure 2. Indirect immunofluorescence in Hep2-positive cells with a homogeneous pattern and a dense fine granular cytoplasmic pattern at a titer of 640.

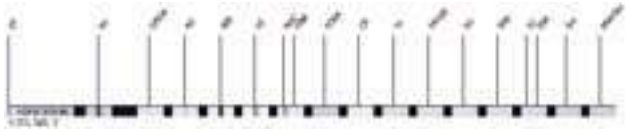
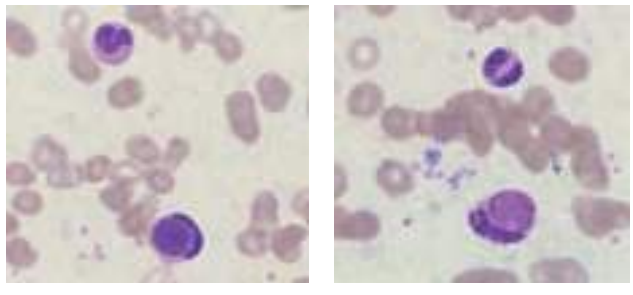


Figure 3. Imunoblot com autoanticorpos anti–histonas, anti-nucleossomas e anti- ribossomal P, positivos.

A skin biopsy was performed, which did not rule out Lupus Erythematosus subacute.
To clarify the bicytopenia and febrile symptoms, a morphological study of the bone marrow was performed. In the bone marrow aspirate (BM), collected in a tube with anticoagulant (EDTA), some LE cells (neutrophils with cytoplasmic inclusions of amorphous nuclear material) were observed (figures 4 and 5) and masses of amorphous nuclear material surrounded by several neutrophils (rosettes) (figures 6 and 7).



Figures 4 and 5. Bone marrow smear with LE cells, neutrophils with cytoplasmic inclusions of amorphous nuclear material. Hematoxylin and eosin staining. 1000X magnification.

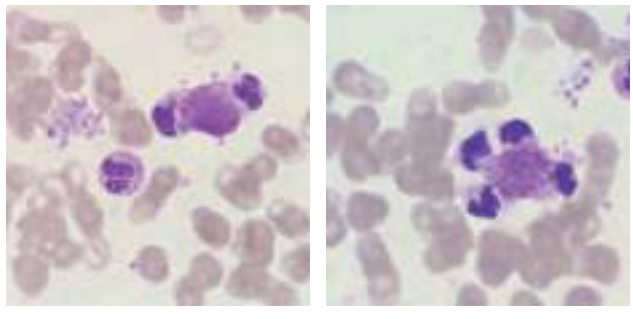


Figura 6 e 7. Esfregaço de sangue medular com massas de material nuclear amorfo rodeadas por vários neutrófilos (rosetas). Coloração Hematoxilina e Eosina. Ampliação 1000X.

DISCUSSION

Systemic Lupus Erythematosus (SLE) is a complex, multisystemic autoimmune disease of varying severity that can present with nonspecific signs and symptoms. In some individuals, skin and osteoarticular changes may predominate, while in others it may affect almost all organs and systems. Due to their variability and complexity of presenta-

tion, the *American College of Rheumatology (ACR)* established in 1982, diagnostic criteria that were subsequently revised in 1997 and further reassessed in 2012 by *Systemic Lupus International Collaborating Clinics*.

The signs and symptoms must be present consecutively or simultaneously:

1. Malar exanthem: a butterfly-shaped rash on the nose and cheek area.
2. Discoid rash: erythema spread throughout the body, including the scalp.
3. Photosensitivity
4. Ulcers in the oral and nasal mucosa, usually painless.
5. Non-erosive arthritis in two or more joints, with edema and intra-articular effusion.
6. Serositis
7. Changes in the nervous system: seizures and/or psychosis
8. Altered renal function: proteinuria > 0.5 g/24 h or cellular casts
9. Hematological abnormalities: hemolytic anemia, leukopenia, or thrombocytopenia.
10. Immunological Changes
• Anti ds-DNA, or
• Anti-Sm, or
• Antiphospholipid antibodies (APAs) based on:
- Abnormal levels of anti-cardiolipin IgG or IgM antibodie
- Positive lupus anticoagulant using standardized method
- False positive serological test confirmed for *Treponema pallidum*, for at least 6 months
11. ANA (antinuclear antibodies) in the absence of pharmacological therapy (1,2).

To confirm the diagnosis of SLE, the presence of 4 or more of these criteria is required (at least 1 clinical and 1 laboratory) or a positive renal biopsy for lupus nephritis with positive ANA's or anti-ds DNA antibody [1,2].

SLE affects individuals of all ages, ethnicities, and genders. However, in more than 75% of cases, patients are female, mostly between 15 and 44 years of age. The most frequent age range for the onset of the disease is between 18 and 55 years, however, it can manifest at any age. Family members of patients with autoimmune diseases have a higher risk of developing the disease, thus there is a familial predisposition [2].

For the initial diagnosis of SLE, the following tests should be requested:

The following laboratory tests:

1. ANA screening by IFI
2. Anti-dsDNA antibodies
3. Anti-Sm antibodies; U1 nRNP; SS-A; SS-B
4. Antiphospholipid antibodies
5. Complement factors C3 and C4, and if both are normal, CH50
6. Other antibodies of interest: Anti-histones, associated with drug-induced lupus), Anti-ribosomal P, associated with neuropsychiatric manifestations, and Anti-nucleosomes [3,4].

The reference method for the study of ANA is indirect immunofluorescence (IFI) on Hep2 cell substrate (cell line derived from humana laryngeal carcinoma cells). These cells, due to their high sensitivity and specificity, allow the observation of a large number of nuclear and cytoplasmic patterns, with emphasis on the study of cellular structures of high decisional importance, such as the high number and dimensions of mitoses [5]. The nuclear pattern described as homogeneous, i.e., with homogeneous distribution of fluorescence throughout the nucleoplasm of Hep2 cells, with marked immunofluorescence of chromosomes on the equatorial plate of the mitotic cell, is associated with the presence of anti-antibodies.

-histones and anti-ds DNA antibodies, which are present in 95% of cases with drug-induced lupus and in 50 to 80% of patients with active or inactive SLE [5].

An LE cell is a leukocyte, almost always a neutrophil, that has phagocytosed denatured nuclear material from another cell as a result of antinuclear activity present in the plasma.

[6]. This phenomenon was first described by Hargraves et al., in bone marrow, in 1948 [7].

CONCLUSION

For decades, prior to autoantibody testing, LE cell research was considered a screening test and the most specific test for diagnosing SLE [8,9]. Although it is currently considered a redundant test with low sensitivity, the observation of LE cells in a bone marrow aspirate, especially in clinical cases not suspected of SLE (notably for clarification of cytopenias), can provide important guidance for diagnosis and should not be underestimated in clinical practice [8,9].

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CASE N.º 10

"Acidental" detection of malaria by the hematology analyzer

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ABSTRACT

We report a clinical case of a 59-year-old woman residing in Angola who presented at the orthopaedic outpatient clinic for gonarthrosis. During preoperative assessment, a CBC revealed a *flag* indicating the presence of parasitised erythrocytes, in addition to atypical cell distribution in the cytogram. An immunochromatographic test confirmed malaria infection, detecting antigens from all human species of the parasite. The blood smear revealed erythrocytes parasitised by *Plasmodium malariae*. When contacted, the attending physician reported that the patient had had a fever in recent weeks and, although he suspected malaria, he did not request specific tests, interpreting the clinical picture as related to gonarthrosis.

INTRODUCTION

Malaria is an infectious disease that causes hundreds of thousands of deaths per year, mainly in endemic areas. In 2018, it is estimated that there were 228 million cases of malaria worldwide and approximately 405,000 deaths that year caused by this infection [1]. The use of new hematological counters can be useful in the diagnosis of malaria, especially in cases where there is no clinical suspicion and in non-endemic areas [2]. Although the peripheral blood smear is the method *gold standard* For diagnosis, the new hematology analyzers are equipped with technology that allows us to suspect the presence of this parasite in the blood through... *flags* and a specific cellular distribution in the cytogram when a complete blood count is performed.

METHOD

A 59-year-old female patient presents to the Orthopedics outpatient clinic at CUF Alvalade for gonarthrosis. A blood count is requested as part of pre-operative analysis. After detection of the pRBC flag [A] (parasitic red blood cell) and visualization of an atypical cell distribution in the cytogram (A) of the blood count, an immunochromatographic test for rapid and qualitative detection of Plasmodium [B] was performed, which was positive for the common antigen (pLDH) to the four Plasmodium species that cause disease in humans (*P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*). In the peripheral blood smear [C], rare erythrocytes parasitized by Plasmodium were observed with morphology suggestive of *Plasmodium malariae* (trophozoites and schizonts).

Figure 1. Hemograma com flag "pRBC"

Figure 2. Immunochromatographic test Malaria

Figura 3. Peripheral blood smear: trophozoites (green arrow) and schizonts (orange arrow) of *Plasmodium malariae*.

The attending physician was contacted, who stated that it was a case of a patient residing in Angola presented with a fever in recent weeks. This condition was initially interpreted as gonarthritis, and although malaria was considered a possibility, no further tests were requested. The patient was referred to the Infectious Diseases Clinic.

DISCUSSION

Since malaria is not endemic in Europe, testing for Plasmodium is only carried out when there is clinical suspicion. Migration flows and increased travel to tropical regions have contributed to a significant increase in malaria cases, so it is essential to have parameters that allow us to consider this diagnostic hypothesis, even when there is no clinical suspicion (the patient often presents with nonspecific symptoms).

Some of the new hematology analyzers (e.g., Sysmex XE-2100) differentiate white blood cells by combining 3 distinct signals: a forward *scatter lightt* (FSC), a side *spreader light* (SSC) and side *fluorescence light* (SFL). The intensity of the FSC indicates the cell volume, while the SSC reflects the complexity and content of the cell (e.g., nucleus and granules). The SFL indicates the amount of DNA and RNA present in the cell. These determinations are made in different "channels" of the hematology analyzer, and in the channel *white blood cell differential* (WDF) we were able to obtain a differentiation of white blood cells with morphological information about them. A careful and detailed observation of the cytogram obtained in the "WDF" channel allows us to suspect the presence of Plasmodium due to the presence of an "abnormal" cell cluster below the neutrophil area and in the eosinophil area [3]. This phenomenon is justified by the fact that in the "WDF" channel the red blood cells are not completely lysed, so even with a reduced number of parasitized erythrocytes, there is an increase in fluorescence in the neutrophil or eosinophil zone, sometimes leading to falsely elevated neutrophil or eosinophil counts. The activation of *flag* (pRBC or iRBC: infected red blood cell) by the hematology analyzer allows obtaining a correct count of neutrophils and eosinophils from the "WNR" channel, avoiding false results due to the presence of trophozoites, gametocytes and/or schizonts [4].

In summary, the presence of an "abnormal" distribution of leukocytes in the cytogram in "WDF" and/or *flags* (pRBC; iRBC) in the blood count may be the first and only signs to suspect a Plasmodium infection.

We conclude, therefore, that the new hematological counters are very important tools in the diagnosis of malaria, representing added value, especially in cases where clinical suspicion is low or absent. However, they only constitute an aid for the Clinical Pathologist, who must confirm the suspicion by observing a peripheral blood smear.

POINTS TO HIGHLIGHT

- Malaria can be diagnosed incidentally in blood tests performed for other reasons, even without prior clinical suspicion, thanks to the capabilities of new hematology analyzers.
- Modern hematology analyzers (such as Sysmex XE-2100) use fluorescence flow cytometry and can detect atypical cell patterns (e.g., *flags* such as pRBC or iRBC) suggestive of Plasmodium sp. infection.
- The presence of an "abnormal" cluster in the WDF cytogram (in the area of eosinophils or below neutrophils) may raise suspicion of malaria, even in samples with few parasitized erythrocytes.
- These changes can lead to erroneous counts elevated

neutrophil or eosinophil counts, which are corrected if the equipment activates the *flag* and resort to the alternative channel (WNR).

• Despite technological advancements, the diagnostic confirmation of malaria still requires direct microscopic observation of a peripheral blood smear by a Clinical Pathologist.

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