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O POSSÍVEL IMPACTO DA INTERFERÊNCIA LABORATORIAL PRÉ-TRANSFUSIONAL
THE POSSIBLE IMPACT OF PRE-TRANSFUSION LABORATORY INTERFERENCE
EL POSIBLE IMPACTO DE LA INTERFERENCIA DE LABORATORIO PREVIA A LA TRANSFUSIÓN

Luís Moura[1] *https://orcid.org/0000-0003-1319-4337*
Liliana Fonseca[1] *http://orcid.org/0000-0001-7294-8077*
Emanuel Rodrigues[1]
Rui Lobo[1]
Arnaldo Brito[1]
Mariana Costa[1]

[1] Unidade Local de Saúde Viseu Dão-Lafões, Viseu, Portugal

Luís Moura – luismfdm@gmail.com | Liliana Fonseca - lilianapvfonseca@gmail.com | Emanuel Rodrigues - rodrigues.emanuel@gmail.com | Rui Lobo - rfilipelobo@gmail.com | Arnaldo Brito - arniemd@hotmail.com | Mariana Costa - moscvv@gmail.com

Corresponding Author:
Luís Moura
Rua do Pessegueiro Lote C
5400-444 –Chaves - Portugal
luismfdm@gmail.com

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RESUMO

Introdução: Os testes pré-transfusionais permitem a deteção de anticorpos clinicamente significativos e a compatibilização prévia de unidades a transfundir.
Objetivo: Descrever um caso clínico tratado com plasmaferese e imunossupressão.
Métodos: Estudo descritivo de um caso clínico de um homem de 55 anos, internado por uma insuficiência renal rapidamente progressiva, tratado com plasmaferese e imunosupressão para redução/remoção do componente patológico imunológico responsável por interferências nos testes pré-transfusionais. Realizou-se monotorização diária da situação clínica com recurso a testes laboratoriais de parâmetros sanguíneos específicos.
Resultados: A diminuição dos títulos de anticorpos, devido à ação dos imunossupressores utilizados em associação com as sessões de TPE, para além do claro impacto clínico na recuperação da função renal, permitiu também remover esta interferência e selecionar as unidades de sangue com maior segurança.
Conclusão: É essencial o desenvolvimento de métodos que identifiquem e removam substâncias patológicas que poderão interferir com a compatibilização de unidades a transfundir, e por isso, com a segurança transfusional.

Palavras-chave: interferência laboratorial; plasmaferese terapêutica; compatibilidade; transfusão

ABSTRACT

Introduction: Pre-transfusion tests allow the detection of clinically significant antibodies and the prior compatibility of units to be transfused.
Objective: To describe a clinical case treated with plasmapheresis and immunosuppression.
Methods: Descriptive study of a clinical case of a 55-year-old man, hospitalized for rapidly progressive renal failure, treated with plasmapheresis and immunosuppression to reduce/remove the pathological immune component responsible for interfering with pre-transfusion tests. Daily monitoring of the clinical situation was carried out using laboratory tests of specific blood parameters.
Results: The decrease in antibody titters, due to the action of the immunosuppressants used in association with the TPE sessions, in addition to the clear clinical impact on the recovery of renal function, also made it possible to remove this interference and select the blood units more safely.
Conclusion: It is essential to develop methods that identify and remove pathological substances that could interfere with the compatibility of units to be transfused and, therefore, with transfusion safety.

Keywords: laboratory interference; therapeutic plasmapheresis; crossmatch; transfusion

RESUMEN

Introducción: Las pruebas pretransfusionales permiten detectar anticuerpos clínicamente significativos y compatibilizar previamente las unidades que se van a transfundir.
Objetivo: Describir un caso clínico tratado con plasmaféresis e inmunosupresión.
Métodos: Estudio descriptivo de un caso clínico de un varón de 55 años, hospitalizado por insuficiencia renal rápidamente progresiva, tratado con plasmaféresis e inmunosupresión para reducir/eliminar el componente inmunológico patológico responsable de interferir en las pruebas pretransfusionales. Se realizó un seguimiento diario de la situación clínica mediante pruebas de laboratorio de parámetros sanguíneos específicos.
Resultados: La disminución de los títulos de anticuerpos, debida a la acción de los inmunosupresores utilizados en asociación con las sesiones de TPE, además de la clara repercusión clínica en la recuperación de la función renal, también permitió eliminar esta interferencia y seleccionar las unidades de sangre con mayor seguridad.
Conclusión: Es fundamental desarrollar métodos que identifiquen y eliminen las sustancias patológicas que puedan interferir en la compatibilidad de las unidades a transfundir y, por tanto, en la seguridad transfusional.

Palabras Clave: interferencia de laboratório; plasmaféresis terapêutica; compatibilidade; transfusión

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INTRODUCTION

A main component of pre-transfusion testing involves mixing the patient's plasma with previously characterized erythrocytes, allowing the detection of clinically significant alloantibodies and/or autoantibodies. This is particularly relevant when it translates into a positive crossmatch that conditions/limits the transfusion conduct.

1. CASE REPORT

Male, 55 years old, vineyard worker, with a personal history of HBP diagnosed about one year ago, T2D NID diagnosed 6 months ago with good metabolic control (HbA1c 6.5% in Jul/23), without target organ damage, associated with overweight and dyslipidemia. Chronic alcoholism (1L wine/day) and ex-smoker 60 pack-year

Referred from PCC due to creatinine elevation on 17/01: 5.80mg/dL. He reported edema of the LLs within about one week of evolution and the appearance of skin lesions on the legs and ankles, which were initially painful. No other rashes. He denies respiratory symptoms, except for fluctuating epistaxis in the last 6 months and sometimes blood-streaked sputum. No recent antibiotic therapy was reported. He denies easy bruising. He reports a perception of decreased urinary output in the previous week, especially during the day, and foamy urine. There is no notion of macroscopic hematuria. Stable tension profile at home. He describes progressive asthenia, skin pallor, and dyspnea for great exertion after 2-3 weeks, which makes it difficult to perform ADLs. Unquantified weight loss associated with anorexia and decreased food intake (reports maintaining water intake). He denies night sweats and fever. He denies nausea, vomiting, abdominal pain, diarrhea, dyspeptic symptoms, and early infarction. One month before, he presented with arthralgias that lasted for 3 weeks with inflammatory rhythm in the knees, hips, and feet and was medicated with naproxen with a resolution of the complaints.

From the complementary study: ANA's negative, RF negative, anti-CCP negative, and VS 112 mm/h. On admission, during the objective examination, there were hematic remnants in the nasal cavities, discolored, slightly dehydrated mucous membranes, and, at the level of the LLs, slight pretibial edema (godet +) and in the anterior tibial region, millimeter erythematous macules that do not disappear on digital pressure. Analytically, anemia NN (9.5g/dL), sCr 8.8mg/dL (Jul/23: 0.77mg/dL), uric acid 10.7 mg/dL and CRP 9.51 mg/dL stand out. In complementary study: FR N, Ig's N, TASO 388. No complement consumption. Anti-MPO antibody negative, AMBG negative; Serum protein electrophoresis with no apparent monoclonal peak; Ac antidsDNS 2(N), ANA and ENA screen negative; Anti PR3 antibody >177 U/mL (N < 10). Active urine sediment: leukocytes 44.9/mL and erythrocytes 135.3/mL. Imaging CT chest of admission with scattered ground glass of central predominance (upper and lower lobes), compatible with alveolar hemorrhage.

On 01/19, he was admitted to nephrology with a diagnosis of PR3-ANCA-associated vasculitis (AAV) with renal, pulmonary, ENT, and cutaneous involvement. He showed a rapidly progressive renal insufficiency (Cr 8.8mg/dL at admission) with sCr 0.77mg/dL baseline (07/23), with petechial/purpuric lesions in the LLs and hematic rhinorrhea with mild hemoptoic cough. During hospitalization, he completed two hemodialysis sessions with good response (CVC Fp was placed on 01/20). Fiber optic bronchoscopy was performed with diagnostic BAL on 01/23, which confirmed DAH. BAL study showed respiratory epithelium and squamous cells without atypia; macrophages, hemosiderophages, and occasional lymphocytes and polymorphonuclear cells; neutrophils in the background with abundant degenerated red blood cells. Therapeutic plasma exchange (TPE) and therapy with Prednisolone and Cyclophosphamide adjusted to renal function were initiated, with improvement in Cr ~3.7-3.9 (stabilized) (see graph 1) while maintaining good diuresis.

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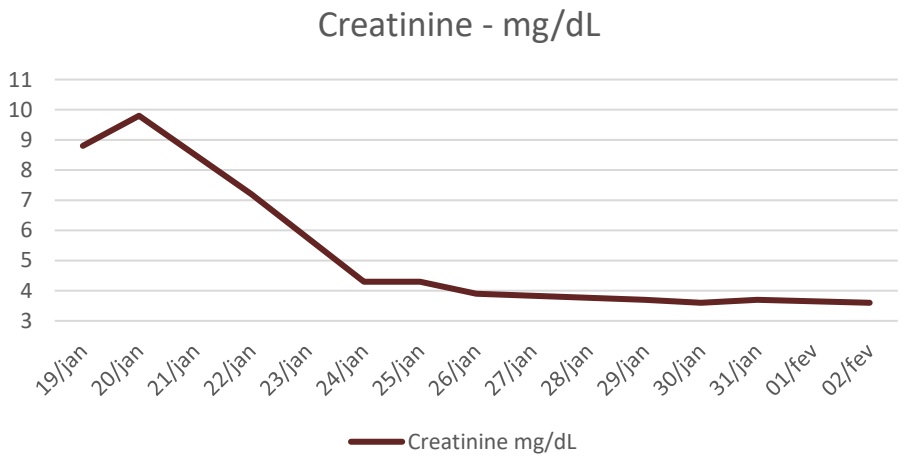
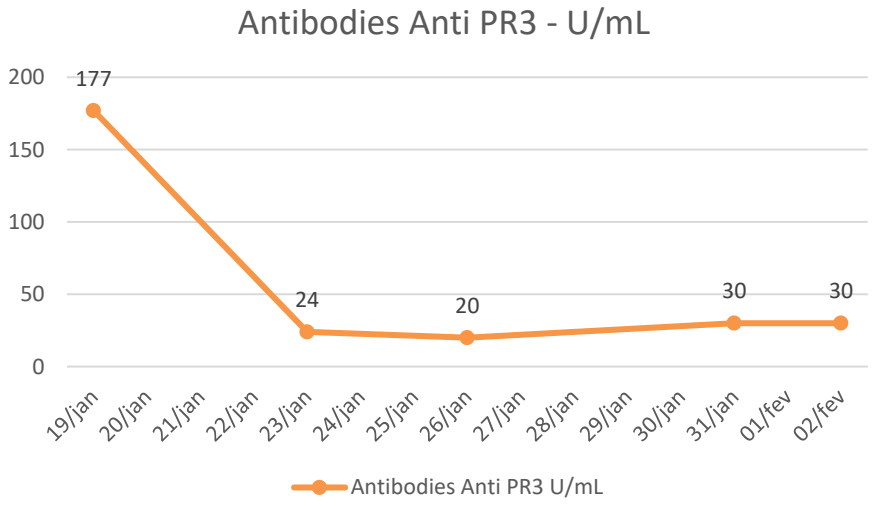


Graph 1

He completed six sessions of daily TPE until 01/26 with progressive reduction of PR3 titers (>177 » 24 » 20 » 30) (see graph 2), sustained even after 7 days from the last session. By isolation of Staphylococcus aureus (MSSA) in the BAL in an immunosuppressed patient, although asymptomatic, he did 7 days of ceftriaxone.



Graph 2

The patient maintained respiratory insufficiency throughout the hospitalization, with resolution of hemoptoic cough and epistaxis. Also during admission the patient presented with decompensated DM in the context of corticosteroid therapy and anemia from vitamin B12 and folic acid deficiency, requiring transfusion 1 concentrated red blood cells on 01/25 due to Hb 6.6g/dL (after 3 doses intravenous iron due to transferrin saturation at the lower limit of normal), under erythropoiesis stimulant agents at maximum dose. This transfusion was delayed for 4 days due to the pan-reactivity demonstrated in the antibody screening and incompatibility (1+) in the crossmatch to all the blood units used. (see graph 3)/ (see gel card results).

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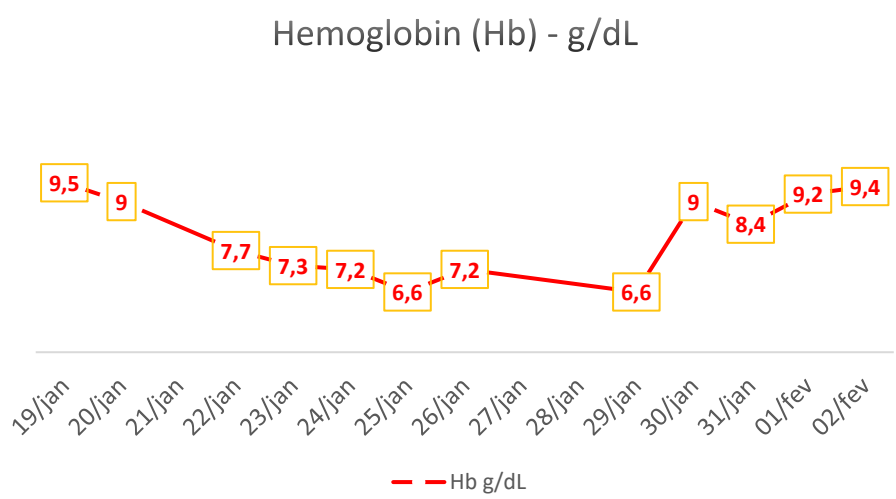

Graph 3

At the date of discharge (D14), he was comfortable, with no complaints, and was sent home with a follow-up appointment for a specialist consultation. However, a week later, he was hospitalized again due to greater fatigue since discharge, de novo appearance peri-orbital edema, worsening of peripheral edema, and tension profile. Despite a slight improvement in renal function during hospitalization, sCr 3.7-4 mg/dL was maintained, assuming a probable baseline value in a patient with a prolonged constitutional condition (secondary to ANCA vasculitis). Thus, due to poor recovery of renal function, with a probable stage 5 CKD with a high risk of progression to terminal CKD in short/medium term, a high hemorrhagic risk associated with uremia, and a high probability of presenting histological lesions compatible with chronicity, it was decided not to proceed with renal biopsy and to maintain immunosuppression. The patient was referred for clarification about the RRT (renal replacement therapy) modalities.
A main component of pre-transfusion testing involves mixing the patient's plasma with previously characterized erythrocytes, allowing the detection of clinically significant alloantibodies and/or autoantibodies. This situation is particularly relevant when it translates into a positive crossmatch that in itself conditions (limits) the transfusion conduct.

Gel Card Results

This methodology is a microtechnique in which cards containing microtubes with a mixture of acrylamide dextran gel particles are used. In some cards, the antiserum is already incorporated (as in the ABO and Rh groupage cards), while others already have AHG serum and an enhancer incorporated. Gel particles act as a filter to trap erythrocyte clumps. In the event of an antigen-antibody reaction, the formed agglutinates are retained in the gel column when the cards are subjected to low centrifugation, determining the pattern of test positivity. The free erythrocytes will settle to the bottom of the microtube.
A positive Direct Antiglobulin Test (DAT) with polyspecific AHG indicates that erythrocytes are sensitized "in vivo" to immunoglobulin and/or complement. To differentiate the reaction, monospecific AHG reagents are used, such as anti-IgG, anti-IgA, anti-IgM and anti-C3c, anti-C3d, suspended in a gel.
Irregular antibody testing involves detecting one or more antibodies by reacting serum or plasma from a sample (or eluted) with cells with a known antigenic profile. Usually, this research is done in an AHG medium (mandatory in terms of pre-transfusion test). Initially, to find irregular antibodies, a panel of 3 cells is used, which can be suspended in a saline medium without any additive (I,II,III cells) or treated with proteolytic enzymes (IP,IIP,IIIP), most often papain.
Whenever this Ab screening is positive, it is necessary to identify (ID) the Ab or Ab(s) responsible for this positivity. These Identification panels must have a variable set of antigens, showing patterns of positivity and negativity, which allow the specificity of the Ab to be inferred. They are usually made up of 11 different erythrocyte suspensions with different phenotypes.
Crossmatch testing consists of putting the recipient's serum in contact with the cells of the potential donor(s) in order to detect antibodies in the recipient that are likely to destroy the donor's cells. In other words, it aims to verify "in vitro" the erythrocyte compatibility between the donor and the recipient. To prepare for a blood transfusion, it is necessary to select the blood component most suitable for the transfusion recipient. Thus, a crossmatch must be preceded by a pre-transfusion study.

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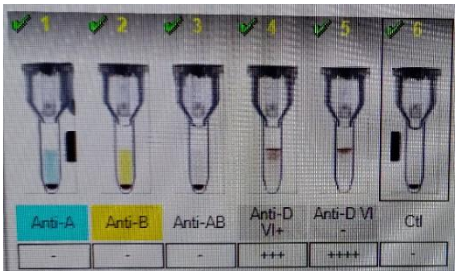
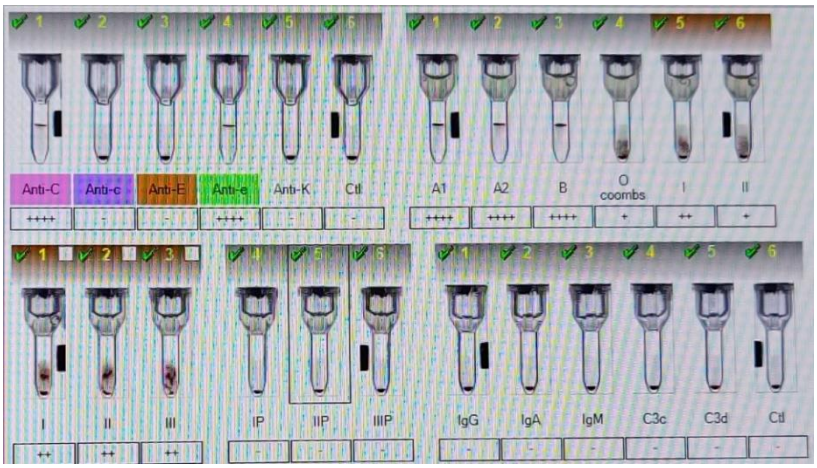
19/01/2024

ABO and Rh testing, Ab screening: *I,II,III* / IP,IIP, IIIP *(Enzyme papanized cells)* and DAT





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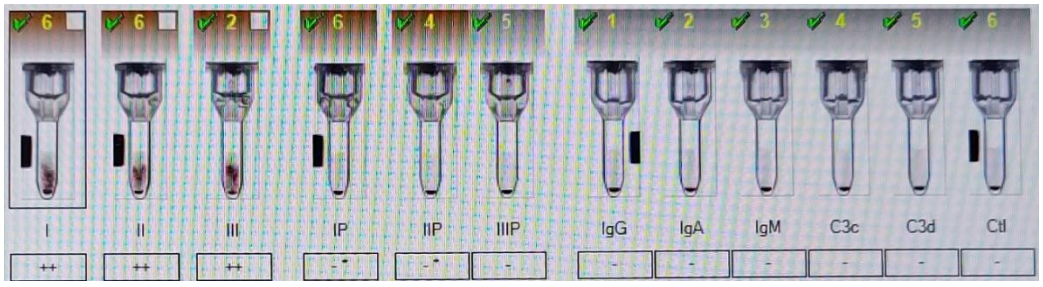
***Ab screening: I,II,III* / IP,IIP, IIIP**



22/01/2024

Identification: 11 test cells + auto-control (AC)

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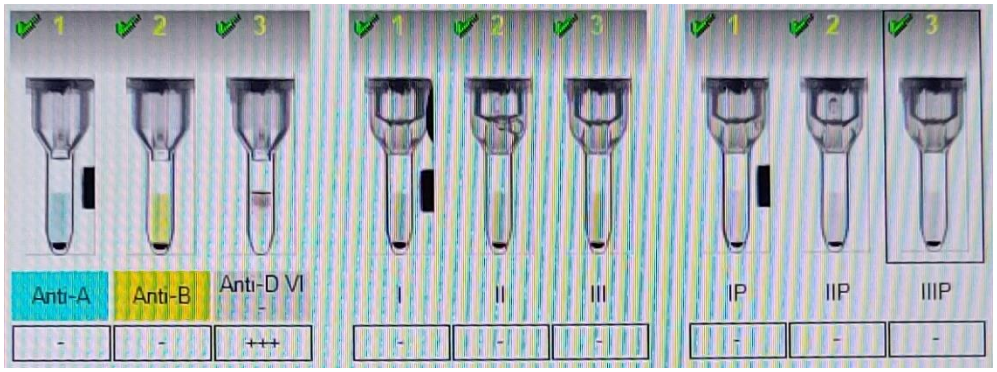
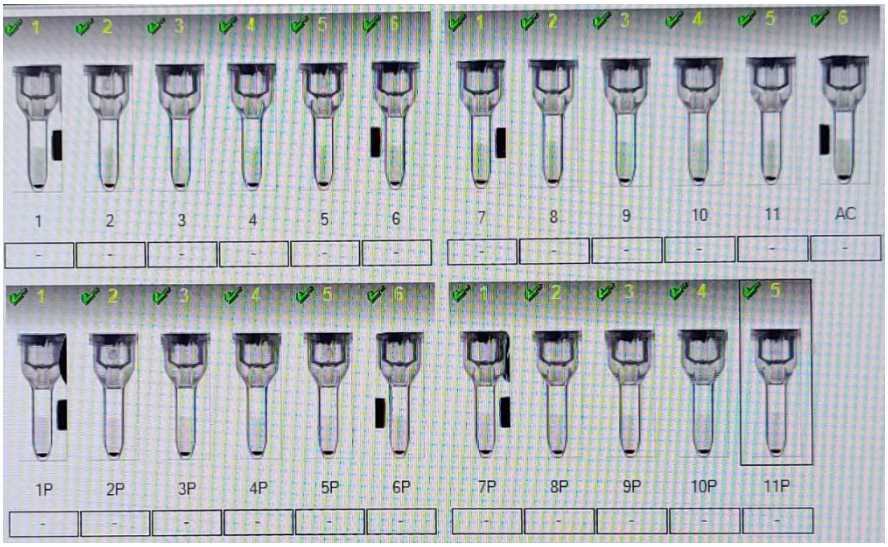
22/01/2024
Crossmatch

25/01/2024
ABO and Rh testing and *Ab screening I,II,III / IP,IIP, IIIP (Enzyme papanized cells)*



25/01/2024
Identification: 11 test cells (P – Enzyme papanized cells)



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25/01/2024
Crossmatch

In the clinical case presented, the first irregular antibody tests were positive in the panel of 3 and 11 cells, demonstrating an interference of pathological antibodies (PR3- ANCA Antibodies), making choosing units with erythrocyte phenotypes compatible difficult in crossmatch. With the removal of this interference, in the pre-transfusion tests carried out on January 25th, we found a negative antibody screening and crossmatch test, finally allowing a transfusion with maximum safety.

CONCLUSION

Developing methods that consistently identify interfering substances and remove or circumvent their origin is critical to identifying clinically significant antibodies. The pre-transfusion interference of these antibodies delays the compatibility of blood units and sometimes even the apheretic treatment of certain pathologies.

In these clinical cases of ANCA-associated vasculitis, the optimum role of apheresis therapy is not established. The decision should be individualized. PEXIVAS was an international RCT that enrolled 704 patients and assessed the effect of TPE on the primary composite outcome of ESKD or death in patients with AAV with an eGFR<50 ml/min or with DAH. Subgroup analysis of patients with Cr ≥5.7 mg/dL or DAH failed to show a statistically significant benefit of plasma exchange. However, a review of complementary data suggested outcomes may favor the TPE groups with DAH and when Cr ≥5.7 mg/dL. Subsequent systematic reviews and meta-analyses, including participants with AAV, found no impact of TPE on all-cause mortality but demonstrated a 20% risk reduction of ESKD at 12 months despite being also associated with an increased risk for serious infections (Walsh, 2022). The American College of Rheumatology (ACR) noted that TPE can be considered for patients at higher risk for progression to ESKD who accept a potential increased risk for infection

In this case, the decrease in antibody titers, due to the action of the immunosuppressants used in association with TPE sessions, in addition to the clear clinical impact on the recovery of kidney function, also allowed this interference to be removed and blood units selected most safely.

LIST OF ABBREVIATIONS

Ab - Antibody
ADLs - activities of daily living
AHG - Anti-human globulin
ANCA - Anti-neutrophil cytoplasm antibodies
AAV - ANCA associated vasculitis
BAL - bronchoalveolar lavage
DAH - Diffuse Alveolar Hemorrhage
DAT - Direct Antiglobulin Test
eGFR - estimated glomerular filtration rate
ENT - Ear, nose and throat
ESKD – End Stage Kidney Disease
T2D NID - Type 2 diabetes, non Insulin dependent
TPE - Therapeutic plasma exchange
CKD - Chronic Kidney Disease
sCr- serum creatinine
CRP - C-reactive protein
CT - Computed Tomography
CVC Fp - Central venous catheters, femoral placement
HbA1c – glycated haemoglobin
HBP - High blood pressure
LLs - lower limbs
MSSA - Methicillin-resistant Staphylococcus aureus
PCC - Primary care center

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AUTHOR CONTRIBUTIONS

Conceptualization, L.M. and R.L.; data curation, L.M., L.F., E.R. and R.L.; formal analysis, R.L., A.B. and M.C.; investigation, L.M.; methodology, L.M. and R.L.; project administration, L.M., L.F. and R.L.; resources, L.M. and R.L.; supervision, R.L., A.B. and M.C.; validation, R.L. and M.C.; visualization, L.M., L.F., E.R. and R.L.; writing-original draft, L.M., L.F., E.R. and R.L.; writing-review and editing, A.B. and M.C.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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