American Journal of Case Reports

e-ISSN 1941-5923
© Am J Case Rep, 2025; 26: e945746
DOI: 10.12659/AJCR.945746

Received: 2024.07.06
Accepted: 2025.02.19
Available online: 2025.02.25
Published: 2025.04.11

Exploring Protein A Immunoadsorption for Autoimmune Hemolytic Anemia with Hyper-IgG4emia

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ACE 1,2 Jianwen Wang
BE 1,2 Yuxin Li
EF 1,2 Xinran Wang
BF 1,2 Xinyu Yang
BD 1,2 Yeyi Yang
AD 1,2 Hao Zhang

1 Department of Nephropathy, Third Xiangya Hospital of Central South University, Changsha, Hunan, PR China
2 The Critical Kidney Disease Research Center of Central South University, Changsha, Hunan, PR China

Corresponding Author: Hao Zhang, e-mail: Zhanghaoliaoqing@163.com
Financial support: Clinical Medical Technology Innovation Guidance Project of Hunan Province (2021SK53712) and the Hunan Province Traditional Chinese Medicine Research Program Project (C2023045)
Conflict of interest: None declared

Patient: Female, 60-year-old
Final Diagnosis: Autoimmune hemolytic anemia (AIHA)
Symptoms: Recurrent dizziness • weakness • darkening of urine and jaundice for 2 months
Clinical Procedure: —
Specialty: General and Internal Medicine

Objective: Unusual or unexpected effect of treatment

Background: Autoimmune hemolytic anemia (AIHA) is a hemolytic anemia characterized by autoantibodies against red blood cells. Patients with AIHA can have 4 subtypes of IgG-type red blood cell antibodies: IgG1, IgG2, IgG3, and IgG4. The development of this disease is closely related to IgG 1 and IgG 3, and the combination with high IgG4 is rare. A patient with autoimmune hemolytic anemia who had a poor response to the steroid combined with immunosuppressive regimen (methylprednisolone and cyclophosphamide) received 4 sessions of protein A immunosorbent therapy with good results and is still under continued follow-up.

Case Report: A 60-year-old woman had recurrent dizziness, weakness, darkening of urine, and jaundice for 2 months. Five years ago, she underwent a lymph node biopsy for "pelvic lymph node enlargement", which indicated "reactive lymph node hyperplasia". Bone marrow aspiration indicated "myelodysplasia", excluding leukemia and plasma cell disease. This patient was first treated with the steroid combination immunosuppressive regimen (methylprednisolone and cyclophosphamide), but she had a poor outcome and an increase in progressive anemia. She was treated with methylprednisolone and cyclophosphamide combined with protein A immunoadsorption therapy. She responded well and her clinical symptoms improved after 2 weeks of treatment. Her malaise was significantly reduced, jaundice decreased, Hb rose to 76 g/L, and IgG4 decreased to 12.4 g/L. At the outpatient review after 2 months, the patient's clinical symptoms had disappeared, hemoglobin (Hb) increased to 136 g/L, and IgG4 decreased to 6.72 g/L.

Conclusions: Protein A immunosorbent therapy may be an effective treatment option for patients with AIHA who have a poor response to conventional therapy.

Keywords: Anemia, Hemolytic • Immunotherapy

Full-text PDF: https://www.amjcaserep.com/abstract/index/idArt/945746

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

Introduction

Autoimmune hemolytic anemia (AIHA) is defined as increased destruction of erythrocytes through autoimmune mechanisms, usually mediated by autoantibodies against erythrocyte surface antigens [1-3]. The most common anti-erythrocyte autoantibody is IgG, which is generally available in 4 serological forms [4,5]. The IgG subclasses involved in the pathogenesis of AIHA are mainly IgG1 and IgG3, which can activate complement cascade reactions, whereas IgG2 and IgG4 are largely uninvolved [6]. However, it has also been shown [7] that the high immunohistochemical reactivity of IgG4 in the vasculature can promote the deposition of other subclasses of IgG, or that IgG4 can induce other autoantibodies, causing inflammatory responses. Therefore, the relationship between elevated IgG4 and AIHA remains unclear and needs to be clarified by further studies.

The first-choice treatment modality for AIHA is glucocorticoids, the second-line treatment is rituximab, splenectomy can be used for those with poor outcomes, and other third-line or follow-up drugs can include azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and bortezomib [1,8]. A case of long-lasting remission of AIHA with staphylococcal protein A, immunosorbent therapy has been reported [9], but its use in the treatment of combined elevated IgG4 has not been reported. Protein A, a membrane protein derived from *Staphylococcus aureus*, has a molecular weight of approximately 42 kDa and contains 5 immunoglobulin-binding sites. This structural feature enables it to effectively interact with immunoglobulins [10]. Staphylococcal protein A immunosorbent is an innovative blood purification therapy that specifically removes IgG antibodies [11]. Considering that AIHA pathogenesis is associated with elevated immunoglobulins, we hypothesized that specific clearance of immunoglobulins could alleviate this disease.

Case Report

A 60-year-old woman had recurrent dizziness, weakness, darkening of urine, and jaundice for 2 months. Five years ago, she underwent a lymph node biopsy for "pelvic lymph node enlargement", which indicated "reactive lymph node hyperplasia".

The patient's initial laboratory evaluation showed hemoglobin 46 g/L, total bilirubin 53.5 umol/L, direct bilirubin 27.1 umol/L, LDH 1073 U/L, EB-DNA quantification 4.404×10^2 copies/ml, direct anti-human globulin test (multiplex) (++), IgG (++), C3d (++), and IgM (+). During the preliminary diagnosis, AIHA was considered. Further examination showed negative cold agglutinin test and platelet antibodies, and negative self-exemption liver set, hepatitis B, hepatitis C, HIV, syphilis, anti-double-stranded DNA antibodies, and ANCA, but was positive for anti-nuclear antibody (1: 320). She had IgG4 75.8 g/L, CRP less than 5 mg/L, and calcitoninogen 0.435 ng/ml. Haptoglobin level was not obtained. Bone marrow smear showed hypoplasia, increased granulocyte fractionated nuclei, increased eosinophils, decreased red lineage, no megakaryocytes, and heaped platelet distribution. Bone marrow biopsy suggested pathological changes in myeloproliferative anemia (**Figure 1**). The results of the bone marrow smear and bone marrow biopsy did not support a diagnosis of leukemia or plasma cell disorders.

CT of the head, chest, abdomen, and pelvis suggested hepatosplenomegaly, chronic inflammation of both lungs, and a small

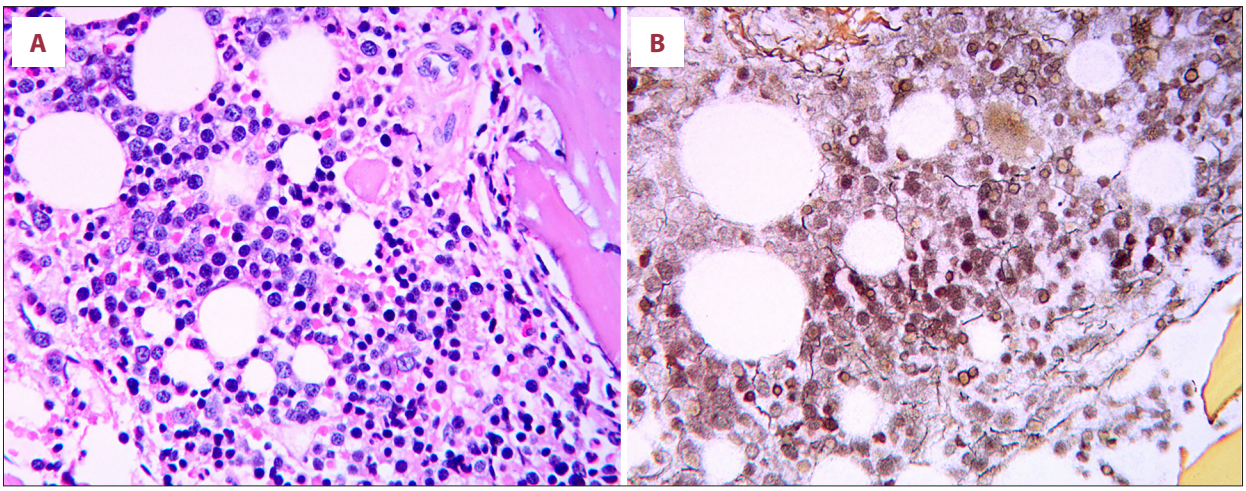

Figure 1. Bone marrow biopsy. (**A**) Bone marrow biopsy (Staining: HE 10×40). (**B**) Bone marrow biopsy (Staining Gomori 10×40). The bone marrow is actively proliferating, and the hematopoietic tissue is proliferating with granulocytes, especially erythrocytes. The ratio of granulocyte to red lineage is reduced, and granulocyte precursor cells are occasionally seen, and the cells of middle and late stages are scattered or in small piles. Lymphocytes and plasma cells were seen. Fibrosis was not seen.

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

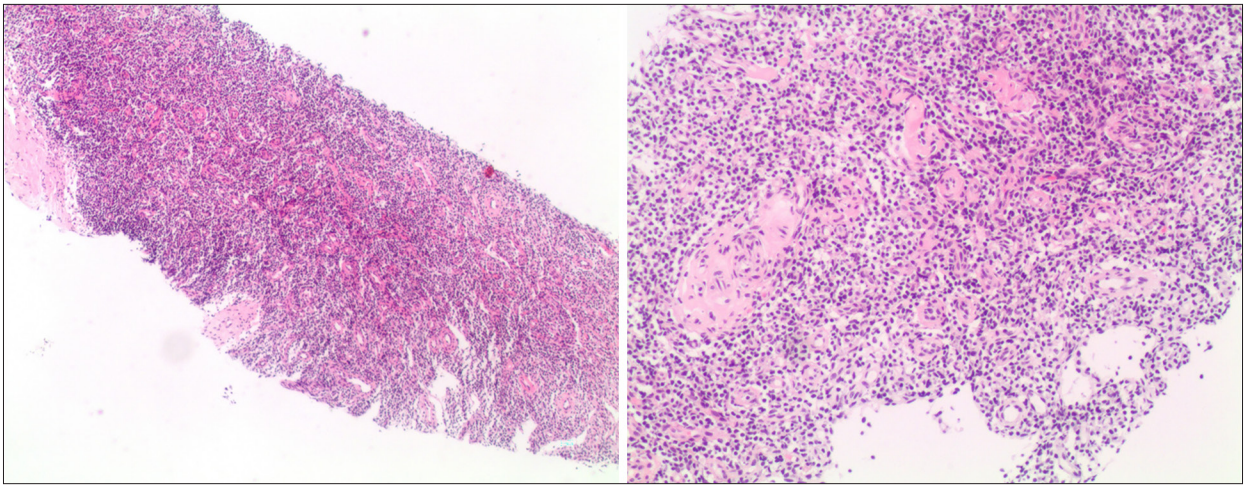

Figure 2. Biopsy of the right inguinal lymph node. Lymphatic tissue hyperplasia with predominant paracortical hyperplasia and small-vessel hyperplasia. Immunohistochemistry: CD3 (+), CD20 (+), CD21 (focal +), Bcl-2 (+), EBER (-), EBER control (+), CD10 (-), Ki-67 (10%), CD5 (+), CD4 (+), CD8 (+), Bcl-6 (-), CXCL-13 (-).

amount of pleural fluid on the right side. There were multiple enlarged lymph nodes in the head and neck, axilla, chest wall, posterior mediastinum, abdominal cavity and retroperitoneum, pelvic parietal iliac vessels, and bilateral inguinal regions. A puncture biopsy of the right inguinal lymph node was considered reactive hyperplasia (**Figure 2**). These finding did not support a diagnosis of lymphoma.

Combined with the above information, the patient was diagnosed with AIHA. She was treated with methylprednisolone 40 mg daily via continuous IV infusion and ceftriaxone for anti-infection therapy. However, her symptoms did not show significant improvement, prompting an increase in the methylprednisolone dose to 80 mg daily via continuous intravenous infusion. Cyclophosphamide 0.4 g was concurrently administered via continuous intravenous infusion. Subsequently, cyclophosphamide 0.4 g was given every other day as a continuous intravenous drip. The retest results showed IgG4 46g/L, hemoglobin (Hb) 50 g/L, erythrocyte pressure product 15.7%, and oxygen saturation 88%. The patient was vomiting. She was first treated with the steroid combination immunosuppressive regimen (methylprednisolone and cyclophosphamide), but had a poor response and an increase in progressive anemia. Staphylococcal protein A immunosorbent can remove IgG antibodies [11]. Considering that AIHA pathogenesis is associated with elevated immunoglobulins, we introduced this treatment option to patients. After fully communicating with the patient, she chose staphylococcal protein A immunosorbent treatment.

Protein A immunoadsorption therapy used a blood purification device (JUN55X, JMS Corporation), a plasma separator (WG-80-PP,ShandongWeigao Blood Purification Products Co., Ltd.), and a protein A immunoadsorption column with a recombinant staphylococcal protein A (KCIA08, Guangzhou Koncen Biotechnology Co.). The immunoadsorption therapy was performed according to the instructions provided in the manual. After establishing extracorporeal circulation using a blood purifier, low-molecular-weight heparin calcium was applied for continuous anticoagulation. The plasma separated by filtration enters the protein A adsorption column through the plasma pump at a flow rate of 30-40 ml/min for adsorption, in one cycle, the adsorption was performed for 15-20 min until 500-600 ml plasma was regenerated. After the plasma in the column was pushed back to the patient with saline, the elution was performed for 7 min to remove the bound antibodies, the equilibration was performed for 7 min to take the column back to neutral, then the column was re-flushed with saline and ready for another cycle. It takes about 40 min for one cycle and 8-10 cycles were performed in a treatment during about 5-6 hours. The patient received a total of 4 treatments, once every other day, and about 5000 ml plasma was regenerated for each treatment. In addition, we collected the eluates from the first and last cycles of the procedure and measured the IgG4 levels. The results showed clinically significant concentrations in the eluates (**Figure 3**), indicating that protein A adsorption is an effective method for removing serum IgG4.

After 2 weeks of treatment, the patient's malaise was significantly reduced, jaundice decreased, Hb rose to 76 g/L, erythrocyte pressure product was 24.3%, total bilirubin was 22.3 umol/L, direct bilirubin was 8.1 umol/L, and IgG4 decreased to 12.4 g/L. The patient was then changed to prednisone tablets 40 mg daily. At the outpatient review after 1 month, the patient's clinical symptoms had resolved significantly, Hb increased to 103g/L, erythrocyte pressure product was 34.1%, total and direct bilirubin returned to normal (**Figure 4**), antinuclear antibody (1: 320) turned negative, and IgG4 continued to be maintained at 12.1g/L. At the outpatient review after 2

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

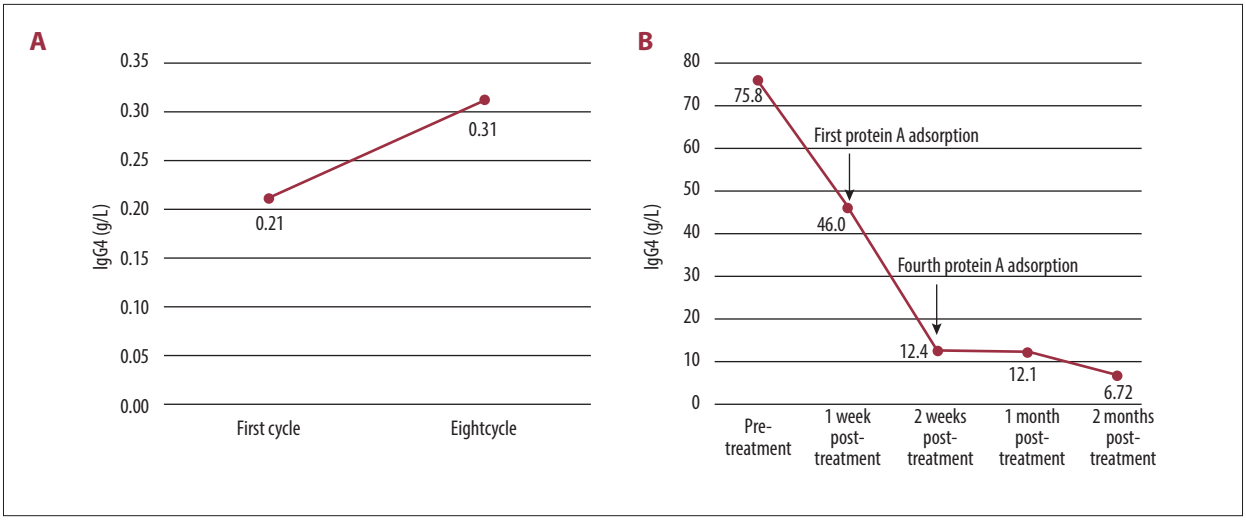

Figure 3. Changes in IgG4 in patient eluates and serum. (**A**) Changes of IgG4 in the eluate between the first cycle and the eighth cycle. (**B**) Changes of serum IgG4 during treatment.

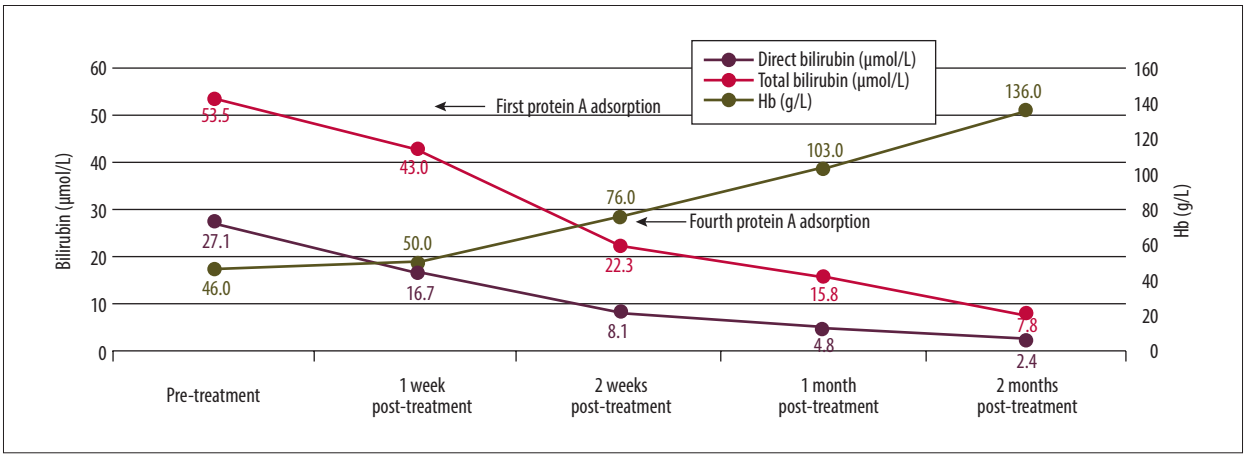

Figure 4. Changes in bilirubin and hemoglobin during treatment.

months, the patient's clinical symptoms had disappeared, Hb increased to 136 g/L, IgG4 decreased to 6.72 g/L (**Figure 3**), total and direct bilirubin were maintained at normal levels, and prednisone was gradually reduced by 20 mg/day.

Discussion

Autoimmune hemolytic anemia (AIHA) is an acquired heterogeneous autoimmune disease characterized by production of antibodies against antigens on autologous red blood cells. Depending on the optimal temperature required for autoantibodies to bind to red blood cells, there are warm, cold antibody types, including cold agglutinin syndrome (CAS) and paroxysmal cold hemoglobinuria (PCH) and mixed types. Warm antibody hemolytic anemia accounts for 65% to 70% of autoimmune hemolytic anemia cases [12]. Our patient fulfilled the criteria for warm antibody type autoimmune hemolytic anemia.

In warm AIHA, autoantibodies are polyclonal (ie, produced by non-clonal B lymphocytes and plasma cells) [1,13]. They are usually of the IgG class, but may also involve IgM warm antibodies and, in rare cases, Ig A warm antibodies 3, [13,14]. IgG subclasses determine extravascular hemolysis mainly through ADCC in the reticuloendothelial system (spleen and, to a lesser extent, liver), affecting the extent to which these antibodies shorten RBC survival. IgG1 is the most common subclass and, together with IgG3, is more effective than IgG2 and IgG4 in shortening the half-life. For complement activation, the IgG3 subclass is more potent, followed by the IgG1 subclass, while the IgG2 and IgG4 subclasses are weaker or ineffective [6]. Our patient was DAT-positive, and although elevated IgG was detected, it was mainly IgG4 that was elevated.

This patient was first treated with the steroid combination immunosuppressive regimen (methylprednisolone and cyclophosphamide), but had a poor outcome and an increase in progressive

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]

anemia, which may be associated with the elevated levels of IgG4. She had generalized multiple lymph node enlargement, and a pathological biopsy suggesting reactive hyperplasia, but no evidence of IgG4-positive plasma cells >10/HPF, IgG4-positive plasma cells to IgG-positive plasma cells ratio >40%, and concomitant fibrosis and occlusive phlebitis were found, so the diagnosis of IgG4-related disease could not be made [15].

A case has been reported where a patient with AIHA experienced a prolonged remission following treatment with staphylococcal protein A immunosorbent therapy [9]. Staphylococcal protein A immunosorbent is an innovative blood purification therapy that specifically removes IgG antibodies. Therefore, we considered this treatment approach. Immunosorbent therapy is a new technology developed from plasma replacement by removing disease-causing factors related to abnormal immune reactions, such as antibodies in plasma, through antigen-antibody immune reactions or by using adsorbent materials to treat the disease. Protein A immunosorbent is a mechanism that specifically binds to human immunoglobulins with the help of staphylococcal protein A (SPA), and the application of bioaffinity chromatography to effectively remove IgG-based (or IgG-type) immunoglobulin pathogenic antibodies from the patient's body by means of extracorporeal circulation. Protein A immunosorbent has the advantage of low impact on blood components and plasma volume, it does not require plasma transfusion, and it reduces the risk of infection or allergic reactions to allogeneic proteins [16].

Thus, we treated our patient with 4 courses of protein A immunosorbent therapy and, interestingly, the patient responded well to clinical symptoms, which suggests that the elevated levels of IgG4 may be associated with the pathogenic role in AIHA. It has been shown that in mice, antibody subclasses that do not activate complement can act synergistically with other immunoglobulin subclasses to activate complement via the lectin pathway. In addition, IgG4 tends to interact with other immunoglobulins [7].The mechanism of this immune response of IgG4 needs further investigation.

Conclusions

We report for the first time that protein A immunosorbent can effectively treat patients with AIHA associated with elevated IgG4 levels who have a poor response to conventional AIHA therapy. This finding offers a new therapeutic approach for this patient population. Protein A immunoadsorbent therapy may be an effective treatment option for patients with AIHA who have a poor response to conventional therapy. Further case-based, multicenter, controlled studies are needed to thoroughly explore the use of protein A immunosorbent therapy in this disease and to investigate its possible mechanisms.

Department and Institution Where Work Was Done

Third Xiangya Hospital of Central South University, Changsha, Hunan, PR China.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

References:

1. Jäger U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: Recommendations from the First International Consensus Meeting. Blood Rev. 2020;41:100648
2. Hill QA, Hill A, Berentsen S. Defining autoimmune hemolytic anemia: A systematic review of the terminology used for diagnosis and treatment. Blood Adv. 2019;3:1897-906
3. Barcellini W, Fattizzo B. The changing landscape of autoimmune hemolytic anemia. Front Immunol. 2020;11:946
4. Quist E, Koepsell S. Autoimmune hemolytic anemia and red blood cell autoantibodies. Arch Pathol Lab Med. 2015;139:1455-58
5. Nailk R. Warm autoimmune hemolytic anemia. Hematol Oncol Clin North Am. 2015;29:445-53
6. Barcellini W. New insights in the pathogenesis of autoimmune hemolytic anemia. Transfus Med Hemother. 2015;42(5):287-93
7. Kawakami T, Yamaguchi N, Soma Y, et al. Immunoglobulin G4-related disease associated with cutaneous vasculitis. Acta Derm Venereol. 2014;94(3):327-28
8. Hill QA, Stamps R, Massey E, et al. The diagnosis and management of primary autoimmune hemolytic anemia. Br J Haematol. 2017;176:395-411
9. Javeed M, Nifong TP, Domen RE, Rybka WB. Durable response to combination therapy including staphylococcal protein A immunoadsorption in life-threatening refractory autoimmune hemolysis. Transfusion. 2002;42(9):1217-20
10. Poullin P, Announ N, Mugnier B, Guis S, et al. Protein A-immunoadsorption (Prosorba column) in the treatment of rheumatoid arthritis. Joint Bone Spine. 2005;72(2):101-103
11. Liu C, Li X, Yang Y, et al. Protein A immunoadsorption for anti-glomerular basement membrane nephritis. Heliyon. 2024;10(15):e35049
12. Liebman HA, Weitz IC. Autoimmune hemolytic anemia. Med Clin North Am. 2017;101(2):351-59
13. Barcellini W, Fattizzo B. How I treat warm autoimmune hemolytic anemia. Blood. 2021;137:1283-94
14. Meulenbroek EM, de Haas M, Brouwer C, et al. Complement deposition in autoimmune hemolytic anemia is a footprint for difficult-to-detect IgM autoantibodies. Haematologica. 2015;100:1407-14
15. Deshpande V. The pathology of IgG4-related disease: Critical issues and challenges. Semin Diagn Pathol. 2012;29(4):191-96
16. Süfke S, Lehnert H, Uhlenbusch-Körwer I, Gebauer F. Safety aspects of immunoadsorption in IgG removal using a single-use, multiple-pass protein A immunoadsorber (LIGASORB): Clinical investigation in healthy volunteers. Ther Apher Dial. 2017;21(4):405-13

Indexed in: [PMC] [PubMed] [Emerging Sources Citation Index (ESCI)] [Web of Science by Clarivate]