

# Complement Blockade with C1 Esterase Inhibitor in Refractory Immune Thrombocytopenia

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## Abstract

Immune thrombocytopenia (ITP) is a disease process that is characterized by anti-platelet autoantibody production and enhanced platelet destruction. Mechanisms implicated in the pathogenesis of ITP include direct damage by cytotoxic T cells, inadequate thrombopoiesis, phagocytosis mediated by autoantibodies and/or complement, an imbalance between effector and regulatory T cells, and communication between T and B cells. The clinical cases presented here describe patients with underlying autoimmune conditions who presented with severe thrombocytopenia. Each patient received standard therapies for ITP including glucocorticoids, intravenous immunoglobulin (IVIG), rituximab, and thrombopoietin receptor agonists with no response. Inadequate responses to all of these therapies do occur, and we propose an alternative mechanism of platelet rescue from peripheral destruction. These patients were deemed to have refractory ITP, and we demonstrate that C1 esterase inhibitor (C1INH) therapy was well tolerated and resulted in platelet count improvement within hours after administration. These findings support the relationship between autoantibody production, complement activation, immune dysregulation, and ultimately, platelet destruction in ITP.

**Key words:** platelet, immune thrombocytopenia, autoantibody, complement, classical pathway, phagocytosis, opsonization, T cells, B cells, C1 esterase inhibitor, therapy.

## Introduction

Immune thrombocytopenia (ITP) is an acquired thrombocytopenia due to autoantibody-mediated destruction of platelets and impaired platelet production.<sup>1</sup> The major autoantibodies implicated in this disease process are directed against platelet membrane glycoproteins, including GPIIb/IIIa and GPIb/IX. The presence of these antibodies has been shown to be related to disease activity.<sup>2,3</sup> Multiple therapies targeting antibody production, the reticuloendothelial system, and platelet production are used to treat ITP, including glucocorticoids,

intravenous immunoglobulin (IVIG), rituximab, splenectomy, Rh0(D) immune globulin, and thrombopoietin receptor agonists. The response to therapy is heterogeneous, supporting the concept that multiple mechanisms are ultimately responsible for thrombocytopenia. Most patients will be treated sequentially with first-line, second-line, and third-line approaches until a safe platelet count is achieved.<sup>4,5</sup>

The mechanisms involved in autoantibody-mediated thrombocytopenia may involve Fc-mediated phagocytosis, C3b-mediated phagocytosis, complement-induced lysis, impaired platelet production, and T-cell cytotoxicity.<sup>6,7</sup> A pathogenic loop model for the ongoing production of anti-platelet autoantibodies in ITP has been proposed.<sup>8</sup> In this model, opsonized platelets are taken up by macrophages in the reticuloendothelial system and present platelet glycoprotein-derived peptides to T cells, which, upon recognition of these peptides, stimulate B cells to make IgG anti-platelet antibodies that bind to circulating platelets, and the cycle continues. It has been shown that ITP patients have increased levels of circulating B cells producing anti-GPIIb/IIIa antibodies.<sup>9</sup>

The complement system is a cascade of proteins involved in innate immune defense and homeostasis.<sup>10,11</sup> In the presence of autoantibodies against platelet antigens, complement is activated via the classical pathway and deposited on the platelet membrane.<sup>12</sup> The presence of antibody and complement on the platelet surface leads to phagocytic uptake in the reticuloendothelial system of the liver and spleen. Additionally, direct damage by C5b-9 (the membrane attack complex) formation on platelet membranes is also thought to contribute to thrombocytopenia in ITP.<sup>13</sup>

C1 esterase inhibitor (C1INH) is a member of the serine protease inhibitor family and interacts with C1 esterase to block activation of the classical pathway of complement.<sup>14</sup> The classical indication for C1INH is hereditary angioedema, and the therapeutic effect of C1INH has been shown in other states of inflammation including, sepsis and ischemia-reperfusion.<sup>15,16</sup>

Case reports have demonstrated that C1INH can prevent C3-mediated lysis of paroxysmal nocturnal hemoglobinuria (PNH) erythrocytes<sup>17</sup> and attenuate hemolysis in a patient with direct antiglobulin test (DAT) C3d positive autoimmune hemo-

lytic anemia.<sup>18</sup> A recent study by Peerschke et al,<sup>19</sup> which included 55 patients with chronic ITP, demonstrated inhibition of the classical pathway of complement *in vitro* with application of a novel C1s inhibitor (TNT003). Based on these data, we hypothesized 1) complement activation/deposition may play an important role in persistent thrombocytopenia in refractory ITP, and 2) blockade of the classical pathway with C1INH may lead to prolonged platelet survival.

### Cases

**Case 1:** A 62-year old woman with a history of Sjogren's syndrome and systemic lupus erythematosus (SLE), presented with a platelet count of 0 (reference range 150,000–450,000) and mild gingival bleeding. Additional labs showed WBC 4000 (4500–11,000), Hgb 9.4 g/dl (12–16 g/dl) (stable from 2 years prior), creatinine 0.76 mg/dl (0.6–1.3 mg/dl), normal liver function tests, LDH 209 U/L (90–190 U/L), and haptoglobin 213 mg/dl (26–185 mg/dl). Laboratory results from 30 days prior to admission revealed a platelet count of 160,000. The diagnosis of ITP was suspected, and treatment with methylprednisolone and IVIG was initiated.

After 2 days of steroids and IVIG, the platelet count remained below 10,000. Bone marrow biopsy was performed that showed trilineage hematopoiesis, including mild megakaryocytic hyperplasia, focal patchy areas of fibrosis, but no evidence of increased blasts. Rituximab was started on hospital day 6. The platelet count did not show any improvement over the next few days (2000–3000), so the dose of steroids was increased, and romiplostim (thrombopoietin receptor agonist) was administered on hospital day 9. The patient continued to receive weekly rituximab, steroids, and an additional dose of romiplostim without significant improvement in the platelet count. She was noted to transiently have a positive DAT C3 on hospital day 2. The C1 esterase inhibitor (Berinert) 20 units/kg was administered intravenously on hospital day 23, and the platelet count increased from 2000 to 12,000 in 6 hours. The patient received two additional doses of C1INH on hospital days 24 and 29. She was discharged on hospital day 30 with a platelet count of 38,000. The platelet count trended up to 105,000 by day 35 and 217,000 one month later and remains normal 20 months post treatment, with no additional intervention.

**Case 2:** A 47-year old woman with a history of Sjogren's syndrome and Raynaud's syndrome, presented with a one week history of petechiae, ecchymoses, and a platelet count of 2000. Other labs included WBC 3000, Hgb 12.3 g/dl, creatinine 0.45 mg/dl, LDH 260 U/L, and haptoglobin <8 mg/dl. The diagnosis of ITP was suspected and prednisone 1 mg/kg was started. As the platelet count remained low (<3000), IVIG was begun on hospital day 4 and was continued for 2 days (total dose 2 g/kg). A rise in platelet count to 90000 was seen on hospital day 7; however, it trended back down to <10,000 on day 11. Bone marrow biopsy was performed that showed normal trilineage hema-

topoiesis with scattered megakaryocytes. Rituximab was initiated on hospital day 12 and was administered weekly for 4 doses. She also received two doses of romiplostim (day 12 and 17), continued on steroids, and was discharged on hospital day 18 with a platelet count of 51,000. Steroids were tapered off (by day after discharge) and the platelet count had risen to 185,000 on day 29.

One week later (day 36 after initial presentation, 3 days after last rituximab), the patient was re-admitted to the hospital with a platelet count of 0. Prednisone 1 mg/kg was restarted, one dose each of IVIG (1 g/kg) and romiplostim were given, and C1INH 20 units/kg was administered on day 37. The platelet count increased from 4000 to 8000 at 8 hours post C1INH. The patient received 2 additional doses of C1INH on day 38 and 39. She was discharged on day 40 with a platelet count of 25,000, which increased first to 122,000, then 469,000 1 week later.

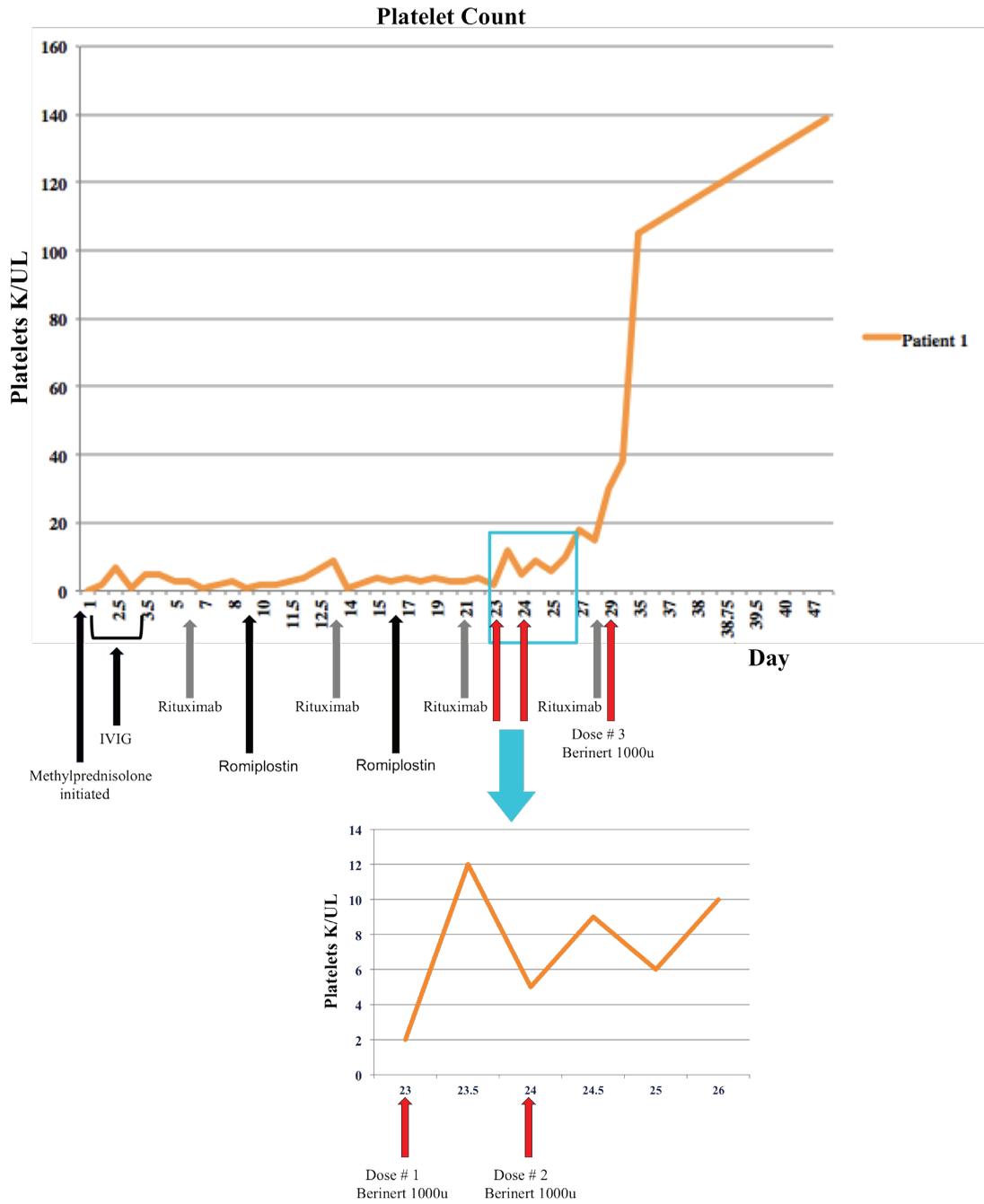
### Discussion

These cases clinically illustrate a potential thought-provoking relationship between platelet destruction and autoantibody production, complement, and the immune system in the pathogenesis of ITP. The exact mechanism of autoimmunity that triggers ITP remains unclear, but has been shown to involve an imbalance between effector and regulatory T cells.<sup>20</sup> Studies in animal models have shown that mice deficient in Tregs exhibit elevated levels of IgG antibodies capable of platelet binding, thus suggesting crosstalk between Tregs and B cells producing IgG anti-platelet autoantibodies.<sup>21,22</sup> Additionally, dysfunction of B regulatory cells (Bregs) in ITP appears to lead to cytokine production, differentiation of Tregs, and reduced function of CD4+ T cells.<sup>20</sup> This interplay between T and B cells likely plays an integral role in the development of ITP.

In a significant portion of ITP patients, platelet autoantibodies have demonstrated the capability of activating the classical pathway of complement. *In vitro* complement fixation assays have shown that serum obtained from 50% of patients with ITP is able to fix complement to the platelet surface.<sup>2</sup> The activation of complement in these cases tends to follow the classical pathway, and autoantibodies to platelet surface antigens GPIIb/IIIa and/or GPIb/IX are major target antigens.<sup>23,24</sup> We suspect that complement fixation/activation plays an important role in platelet destruction in ITP. Proposed mechanisms include C3b deposition on the platelet surface leading to opsonization, direct damage to platelet membrane by C5b-9, and/or a role for complement in the imbalance in T-cell regulator/effector activity.

We suspect a potential biphasic response to C1INH therapy. We hypothesize immediate inhibition of the classical pathway and subsequent decrease of C3b deposition on platelet surface, C3b-mediated phagocytosis, and/or C5b-9-mediated membrane damage may be responsible for the acute rise in platelet count, while a reset of T cell regulatory/effector function via complement blockade may account for the longevity of platelet count increase and normalization seen in our patients. We also suspect

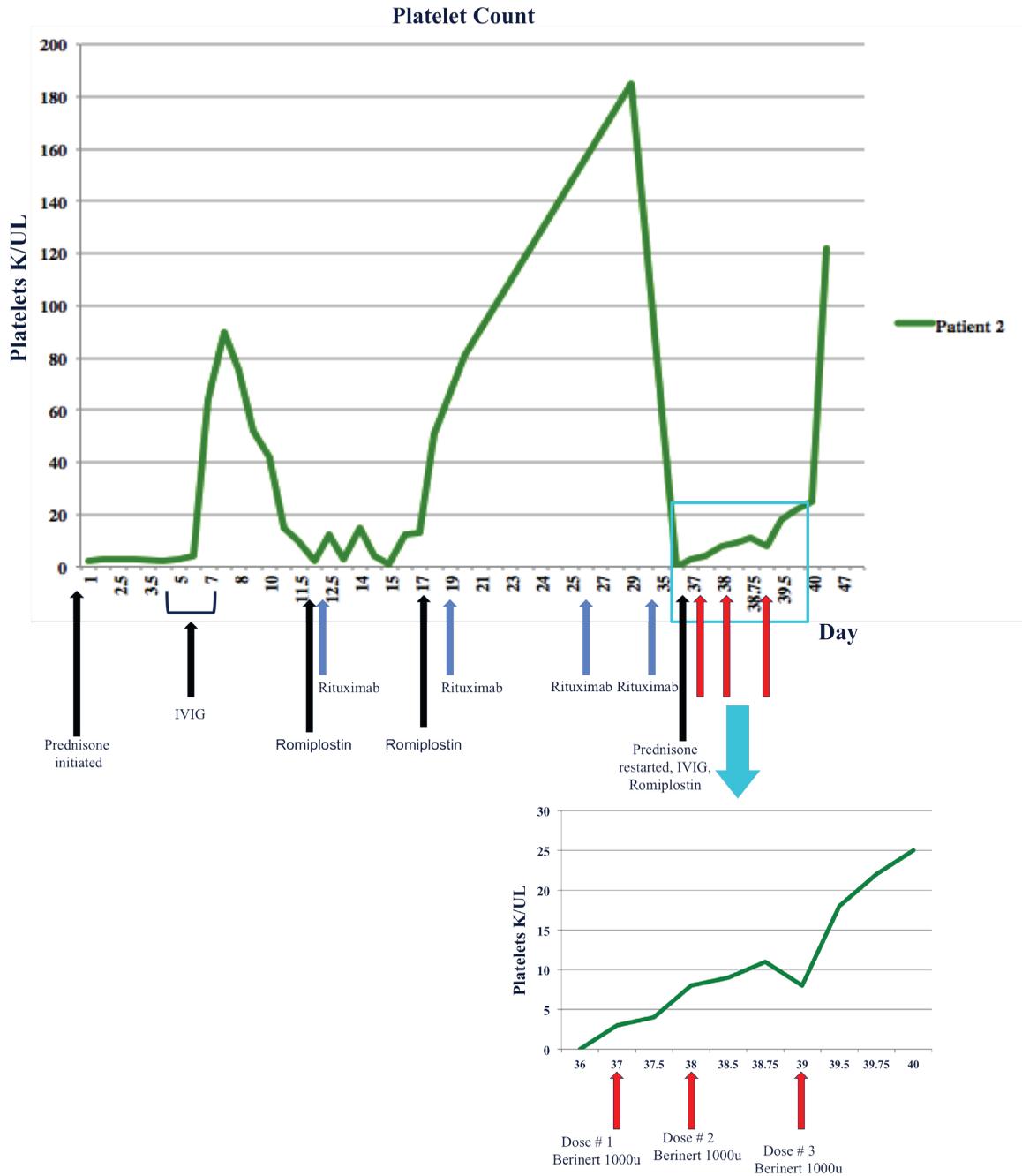
FIGURE 1A.



Platelet count trends for patient 1, with magnified views of time points with Berinert was administered. For patient 1, C1INH (Berinert) was administered on hospital days 23, 24, and 29. Both patients also received steroids, IVIG, weekly rituximab for 4 doses, and thrombopoietin receptor agonist as treatments during clinical course.

IVIG indicates intravenous immunoglobulin.

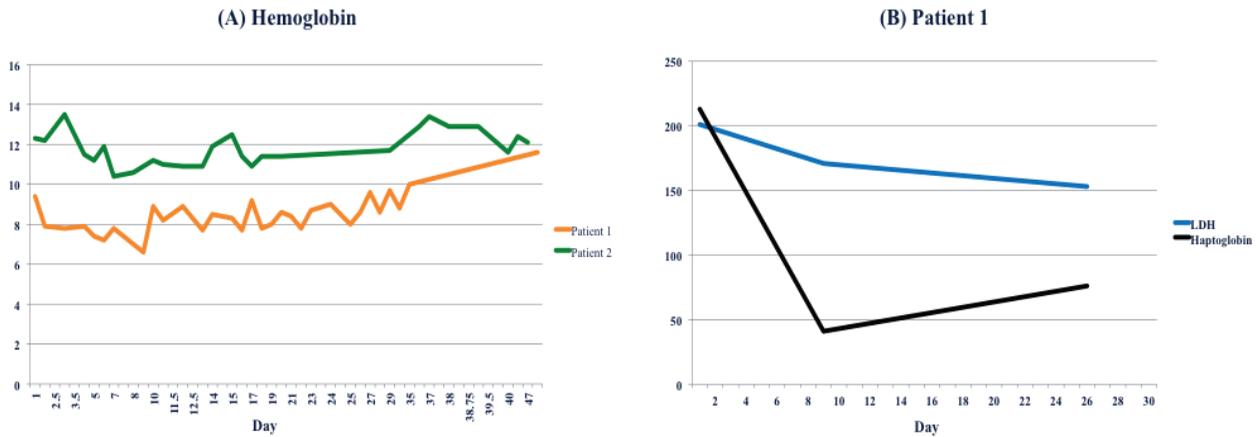
FIGURE 1B.



Platelet count trends for patient 2, with magnified time points when Berinert was administered. For patient 2, C1INH (Berinert) was given on hospital days 37, 38, and 39. Both patients also received steroids, IVIG, weekly rituximab for 4 doses, and thrombopoietin receptor agonist as treatments during clinical course.

IVIG indicates intravenous immunoglobulin.

**FIGURE 2.**



Time trend in hemoglobin levels for patients 1 and 2 (A) and LDH and haptoglobin trend for patient 1 (B), which demonstrate evidence of decreased hemolysis over time. Y-axis in (A) represents mg/dl, y-axis in (B) represents U/L and mg/dl for LDH and haptoglobin curves, respectively.

modulation of signaling between T and B cells, affecting ongoing antibody production. The chronic disease course for some ITP patients may be due to underlying infections, stress events, or polymorphisms that contribute to persistent activation of complement and the immune system.

**Conclusion**

In our patients, the commercially available C1INH, Berinert, was well tolerated and platelet count improvement was noted almost immediately after administration and has appeared to be sustained. A better understanding of the underlying mechanisms of platelet destruction in ITP may help to better define the disease and individualize therapy for patients who do not appropriately respond to standard therapies. Future studies evaluating treatments that target inhibition of the complement pathway may be an effective alternative or adjunctive therapy for refractory immune thrombocytopenia.

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**REFERENCES**

1. Cines D, Blanchette VS. Immune thrombocytopenic purpura. *N Engl J Med.* 2002;346(13):995-1008.
2. Najaoui A, Bakchoul T, Stoy J, et al. Autoantibody-mediated

- complement activation on platelets is a common finding in patients with immune thrombocytopenic purpura (ITP). *Eur J Haematol.* 2011;88:167-174. doi: 10.1111/j.1600-0609.2011.01718.x.
3. Berchtold P, Wenger M. Autoantibodies against platelet glycoproteins in autoimmune thrombocytopenic purpura: their clinical significance and response to treatment. *Blood.* 1993;81(5):1246-1250.
4. Ghanima W, Godeau B, Cines DB, Bussel JB. How I treat immune thrombocytopenia: the choice between splenectomy or a medical therapy as a second-line treatment. *Blood.* 2012;120(5):960-969. doi: 10.1182/blood-2011-12-309153.
5. Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood.* 2011;117(16):4190-4207. doi: 10.1182/blood-2010-08-302984.
6. McMillan R. The pathogenesis of chronic immune thrombocytopenia purpura. *Semin Hematol.* 2007;44:S3-S11. doi: 10.1053/j.seminhematol.2007.11.002.
7. McFarland J. Pathophysiology of platelet destruction in immune (idiopathic) thrombocytopenic purpura. *Blood Rev.* 2002;16(1):1-2.
8. Kuwana M, Okazaki Y, Ikeda Y. Splenic macrophages maintain the anti-platelet autoimmune response via uptake of opsonized platelets in patients with immune thrombocytopenic purpura. *J Thromb Haemost.* 2009;7(2):322-329. doi: 10.1111/j.1538-7836.2008.03161.x.
9. Chen J, Yang L, Chang L, Feng JJ, Liu JQ. The clinical significance of circulating B cells secreting anti-glycoprotein IIb/IIIa antibody and platelet glycoprotein IIb/IIIa in patients with primary immune thrombocytopenia. *Hematology.* 2012;17(5):283-290. doi: 10.1179/1607845412Y.0000000014.

10. Carroll MC. The complement system in regulation of adaptive immunity. *Nat Immunol*. 2004;5(10):981-986.
11. Verschoor A, Langer HF. Crosstalk between platelets and the complement system in immune protection and disease. *Thromb Haemost*. 2013;110(5):910-919. doi: 10.1160/TH13-02-0102.
12. McMillan R. Autoantibodies and autoantigens in chronic immune thrombocytopenic purpura. *Semin Hematol*. 2000;37(3):239-248.
13. Kurata Y, Curd J, Tamerius J, McMillan R. Platelet-associated complement in chronic ITP. *Br J Haematol*. 1985;60(4):723-733.
14. Zeerleder S. C1-Inhibitor : More than a serine protease inhibitor. *Semin Thromb Hemost*. 2011;37:362-374. doi: 10.1055/s-0031-1276585.
15. Liu D, Lu F, Qin G, Fernandes SM, Li J, Davis AE 3rd. C1 inhibitor-mediated protection from sepsis. *J Immunol*. 2007;179(6):3966-3972.
16. Horstick G, Heimann A, Gotze O, et al. Intracoronary application of C1 esterase inhibitor improves cardiac function and reduces myocardial necrosis in an experimental model of ischemia and reperfusion. *Circulation*. 1997;95(3):701-708.
17. DeZern AE, Uknis M, Yuan X, et al. Complement blockade with a C1 esterase inhibitor in paroxysmal nocturnal hemoglobinuria. *Exp Hematol*. 2014;42(10):857-861. doi: 10.1016/j.exphem.2014.06.007.
18. Wouters D, Femke S, Strengers P, et al. C1-esterase inhibitor concentrate rescues erythrocytes from complement-mediated destruction in autoimmune hemolytic anemia. *Blood*. 2013;121(7):1242-1244. doi: 10.1182/blood-2012-11-467209.
19. Peerschke EIB, Panicker S, Bussel J. Classical complement pathway activation in immune thrombocytopenia purpura: inhibition by a novel C1s inhibitor. *Br J Haematol*. 2016;173(6):942-945. doi: 10.1111/bjh.13648.
20. McKenzie CGJ, Guo L, Freedman J, Semple JW. Cellular immune dysfunction in immune thrombocytopenia (ITP). *Br J Haematol*. 2013;163(1):10-23. doi: 10.1111/bjh.12480.
21. Nishimoto T, Kuwana M. CD4+CD25+Foxp3+ regulatory T cells in the pathophysiology of immune thrombocytopenia. *Semin Hematol*. 2013;50 Suppl 1(1):S43-S49. doi: 10.1053/j.seminhematol.2013.03.018.
22. Nishimoto T, Satoh T, Simpson EK, Ni H, Kuwana M. Predominant autoantibody response to GPIb/IX in a regulatory T-cell-deficient mouse model for immune thrombocytopenia. *J Thromb Haemost*. 2013;11(2):369-372. doi: 10.1111/jth.12079.
23. Peerschke EIB, Andemariam B, Yin W, Bussel JB. Complement activation on platelets correlates with a decrease in circulating immature platelets in patients with immune thrombocytopenic purpura. *Br J Haematol*. 2010;148(4):638-645. doi: 10.1111/j.1365-2141.2009.07995.x.
24. Bakchoul T, Walek K, Krautwurst A, et al. Glycosylation of autoantibodies: Insights into the mechanisms of immune thrombocytopenia. *Thromb Haemost*. 2013;110(6):1259-1266. doi: 10.1160/TH13-04-0294.