

Clinical Roundtable Monograph

Clinical Advances in Hematology & Oncology

November 2012

Paroxysmal Nocturnal Hemoglobinuria: A Case-Based Discussion

Moderator



Jeff Szer, MBBS, FRACP
Professor/Director
Department of Clinical Haematology & BMT Service
Royal Melbourne Hospital
Melbourne, Australia

Discussants



Anita Hill, MBChB (Hons), MRCP, FRCPath, PhD
Consultant Haematologist and Honorary Senior Lecturer
Department of Haematology
St James' Institute of Oncology
Leeds Teaching Hospitals
Leeds, United Kingdom



Ilene Ceil Weitz, MD
Associate Professor of Clinical Medicine
Keck-USC School of Medicine
Los Angeles, California

Abstract: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired disorder characterized by chronic intravascular hemolysis as the primary clinical manifestation and morbidities that include anemia, thrombosis, renal impairment, pulmonary hypertension, and bone marrow failure. The prevalence of the PNH clone (from <1–100% PNH granulocytes) is approximately 16 per million, and careful monitoring is required. The average age of onset of the clinical disease is the early 30s, although it can present at all ages. PNH is caused by the acquisition of a somatic mutation of the gene phosphatidylinositol glycan anchor (PIG-A) in a multipotent hematopoietic stem cell (HSC), with clonal expansion of the mutated HSC. The mutation causes a deficiency in the synthesis of glycosylphosphatidylinositol (GPI). In cells derived from normal HSCs, the complement regulatory proteins CD55 and CD59 are anchored to the hematopoietic cell membrane surface via GPI, protecting the cells from complement-mediated lysis. However, in patients with PNH, these 2 proteins, along with numerous other GPI-linked proteins, are absent from the cell surface of red cells, granulocytes, monocytes, and platelets, resulting in complement-mediated intravascular hemolysis and other complications. Lysis of red blood cells is the most obvious manifestation, but as other cell lineages are also affected, this complement-mediated attack contributes to additional complications, such as thrombosis. Eculizumab, a humanized monoclonal antibody against the C5 complement protein, is the only effective drug therapy for PNH patients. The antibody prevents cleavage of the C5 protein by C5 convertase, in turn preventing generation of C5b-9 and release of C5a, thereby protecting from hemolysis of cells lacking the CD59 surface protein and other complications associated with complement activation. Drs. Ilene C. Weitz, Anita Hill, and Jeff Szer discuss 3 recent cases of patients with PNH.



EDITORIAL ADVISORY BOARD

Editor-in-Chief

Bruce D. Cheson, MD
Georgetown University Hospital
Lombardi Comprehensive
Cancer Center

Section Editors

Oncology

Clifford A. Hudis, MD
Memorial Sloan-Kettering Cancer
Center and Weill Medical College
of Cornell University

Mark J. Ratain, MD
The University of Chicago

Hematologic Malignancies

Susan O'Brien, MD
The University of Texas
M. D. Anderson Cancer Center

Hematology

Craig M. Kessler, MD
Georgetown University
Medical School
Lombardi Comprehensive
Cancer Center

David B. Agus, MD
University of Southern California
Keck School of Medicine

Kenneth C. Anderson, MD
Dana-Farber Cancer Institute

James R. Berenson, MD
Institute for Myeloma
& Bone Cancer Research

Howard A. Burris III, MD
The Sarah Cannon
Cancer Center

John Byrd, MD
Ohio State University
Comprehensive Cancer Center

Mitchell S. Cairo, MD
Columbia University

George P. Canellos, MD
Dana-Farber Cancer Institute
Harvard Medical School

Michael A. Carducci, MD
The Sidney Kimmel
Comprehensive Cancer Center
at Johns Hopkins

Edward Chu, MD
University of Pittsburgh

Bertrand Coiffier, MD
Hospices Civils de Lyon
Centre Hospitalier Lyon-Sud

Jeffrey Crawford, MD
Duke University
Medical Center

Myron S. Czuczman, MD
Roswell Park Cancer Institute
SUNY at Buffalo, School of Medicine

David C. Dale, MD
University of Washington

George D. Demetri, MD
Dana-Farber Cancer Institute
Harvard Medical School

Lee M. Ellis, MD
The University of Texas
M. D. Anderson Cancer Center

Elihu H. Estey, MD
Fred Hutchinson Cancer Center

David S. Ettinger, MD
The Sidney Kimmel
Comprehensive Cancer Center
at Johns Hopkins

Robert A. Figlin, MD
Cedars-Sinai Comprehensive
Cancer Center

Charles Fuchs, MD, MPH
Dana-Farber Cancer Institute

Daniel J. George, MD
Duke University Medical Center

Andre Goy, MD
Hackensack University
Medical Center

William Gradishar, MD
Northwestern University

Richard J. Gralla, MD
North Shore University Hospital
Long Island Jewish Medical Center

F. Anthony Greco, MD
The Sarah Cannon Cancer Center

Stephanie A. Gregory, MD
Rush Medical College
Rush University Medical Center

Stuart A. Grossman, MD
The Sidney Kimmel
Comprehensive Cancer Center
at Johns Hopkins

Axel Grothey, MD
Mayo Clinic, Rochester

John D. Hainsworth, MD
The Sarah Cannon Cancer Center

Roy S. Herbst, MD, PhD
Yale Cancer Center

Steven M. Horwitz, MD
Memorial Sloan-Kettering
Cancer Center

Sundar Jagannath, MD
Mount Sinai Medical Center

David H. Johnson, MD
University of Texas
Southwestern Medical Center

Brad S. Kahl, MD
University of Wisconsin

Hagop M. Kantarjian, MD
The University of Texas
M. D. Anderson Cancer Center

Neil E. Kay, MD
Mayo Clinic

John M. Kirkwood, MD
University of Pittsburgh
Cancer Institute

Corey J. Langer, MD, FACP
University of Pennsylvania
Hematology-Oncology Division

Richard A. Larson, MD
University of Chicago

Jeffrey C. Laurence, MD
New York Presbyterian Hospital
Weill Cornell Medical College

John P. Leonard, MD
Weill Medical College
of Cornell University
New York Presbyterian Hospital

Maurie Markman, MD
Cancer Treatment Centers
of America

John L. Marshall, MD
Georgetown University

Kathy D. Miller, MD
Indiana University
School of Medicine

Ruth O'Regan, MD
Winship Cancer Institute
Emory University

Edith A. Perez, MD
Mayo Clinic, Jacksonville

Marshall R. Posner, MD
Dana-Farber Cancer Institute
Harvard Medical School

Paul G. Richardson, MD
Harvard Medical School
Dana-Farber Cancer Institute

Hope S. Rugo, MD
University of California San Francisco
Medical Center

Leonard Saltz, MD
Memorial Sloan-Kettering
Cancer Center

Alan B. Sandler, MD
Oregon Health & Science
University

Charles A. Schiffer, MD
Karmanos Cancer Institute
Wayne State University
School of Medicine

Richard L. Schilsky, MD
University of Chicago

Lee Schwartzberg, MD
The West Clinic

David S. Siegel, MD, PhD
Hackensack University Medical Center

George W. Sledge Jr., MD
Indiana University
Cancer Center

Mark A. Socinski, MD
University of Pittsburgh School
of Medicine

Margaret Tempero, MD
University of California,
San Francisco Comprehensive
Cancer Center

Alan P. Venook, MD
University of California,
San Francisco Comprehensive
Cancer Center

Everett E. Vokes, MD
University of Chicago

Peter H. Wiernik, MD
St. Luke's-Roosevelt
Hospital Center

John R. Wingard, MD
University of Florida
College of Medicine

Clinical Advances in

HEMATOLOGY & ONCOLOGY[™]
A Peer-Reviewed Journal

CEO and Co-Publisher

Steven H. Kurlander

President and Co-Publisher

Paul H. McDaniel

Editorial Director

Jacquelyn Matos

Editor

Stacey Small

Art Director

Derek Oscarson

**Indexed in Index Medicus/MEDLINE/PUBMED
and EMBASE**

Postmaster: Please send address changes (form 3579) to *Clinical
Advances in Hematology & Oncology* c/o DMD, 2340 River Road,
Des Plaines, IL 60018.

ISSN: 1543-0790

Table of Contents

Paroxysmal Nocturnal Hemoglobinuria: Case 1 Ilene Ceil Weitz, MD	4
Paroxysmal Nocturnal Hemoglobinuria: Case 2 Anita Hill, MBChB (Hons), MRCP, FRCPath, PhD	8
Paroxysmal Nocturnal Hemoglobinuria: Case 3 Jeff Szer, MBBS, FRACP	11
Slide Library	14

Disclaimer

Funding for this clinical roundtable monograph has been provided by Alexion Pharmaceuticals, Inc. Support of this monograph does not imply the supporter's agreement with the views expressed herein. Every effort has been made to ensure that drug usage and other information are presented accurately; however, the ultimate responsibility rests with the prescribing physician. Millennium Medical Publishing, Inc., the supporter, and the participants shall not be held responsible for errors or for any consequences arising from the use of information contained herein. Readers are strongly urged to consult any relevant primary literature. No claims or endorsements are made for any drug or compound at present under clinical investigation.

©2012 Millennium Medical Publishing, Inc., 611 Broadway, Suite 310, New York, NY 10012. Printed in the USA. All rights reserved, including the right of reproduction, in whole or in part, in any form.

Paroxysmal Nocturnal Hemoglobinuria: Case 1

Ilene Ceil Weitz, MD
Associate Professor of Clinical Medicine
Keck-USC School of Medicine
Los Angeles, California

The patient was a 31-year-old man who noted worsening fatigue for a year prior to presentation. He reported that he could not think clearly, and he was tired all the time. He had been a high-caliber chess player, and his capacity to play chess was diminished. In June 2011, he developed a high fever and cough that led to a diagnosis of left lower lobe pneumonia. On presentation to the hospital, his blood tests showed the following: hemoglobin of 6.7 g/dL, white blood cell count of 6,800 cells/ μ L, granulocyte count of 4,500 cells/ μ L, platelets of 172,000 per μ L, reticulocyte count of 5.4%, lactate dehydrogenase (LDH) of 1,925 U/L, negative direct Coombs test, haptoglobin of less than 12 mg/dL, and D-dimers greater than 5,000 μ g/L. A bone marrow biopsy showed normal cytogenetics and a hypercellular marrow. Because of the suggestion of hemolysis, flow cytometry was performed and showed 9.9% type II and 9.5% type III red blood cells, or 19.4% combined, after the patient received a blood transfusion. His glycosylphosphatidylinositol (GPI)-deficient granulocyte and monocyte clones were 94.6% and 92.5%, respectively, consistent with the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH).

Discussion

Jeff Szer, MBBS, FRACP I understand your interest in the D-dimers, which may indicate increased risk of thrombosis, but why were levels assessed as part of the initial diagnosis?

IW Testing was performed at an outside hospital. However, at my hospital, we probably would also have performed the test because the patient was hemolyzing. A pulmonary embolus (PE) was in the differential diagnosis, as well as pneumonia. Given his subsequent diagnosis of PNH, we would have ordered them as a routine. Prior to the availability of eculizumab, we would have checked D-dimers in order to decide if the patient needed prophylactic anticoagulation.

JS I gather that the trigger for examining the blood via flow cytometry was the hypercellular bone marrow.

IW Correct! Clearly, he had evidence of hemolysis—his haptoglobin was low and his reticulocyte count was high—but his Coombs test was negative, suggesting unexplained intravascular hemolysis.

JS Could you please discuss the triggers for investigation?

IW The patient's reticulocyte count was high, at 5.4% corrected. His high LDH, negative Coombs test, and low haptoglobin all suggested a non-Coombs-mediated hemolytic process.

JS How was the patient managed?

IW A flow cytometry was performed, which was consistent with the diagnosis of PNH. The discrepancy between the red blood cell and white blood cell clones was due to brisk hemolysis and transfusion. Given the large white cell clone, the patient was then started on eculizumab, with rapid and significant improvement in his fatigue. As described by Weitz and colleagues, PNH patients experience an extensive burden of fatigue with impaired quality of life.¹ In their study of 29 PNH patients, 96% complained of fatigue, 76% were forced to modify their daily activities to manage their PNH, and 17% were unemployed due to the disease. Notably, symptoms were not related to anemia or transfusion requirements. Fifty-nine percent of the patients had not received a transfusion in over 2 years.

JS Did his chess rankings improve?

IW They did. The patient is back at work, traveling, and completely functional. He has no evidence of significant end-organ damage.

JS What was the effect on the D-dimers?

Table 1. Effect of Eculizumab on Markers of Inflammation (IL-6)

9 of 11 patients had elevated IL-6 levels pretreatment. (The 2 who did not were receiving prednisone.)

During the 4-week induction phase of treatment, there was a statistically significant decrease in:

- LDH ($P < .0001$)
- D-dimers ($P = .0057$)
- TAT ($P = .0138$)
- IL-6 ($P = .0362$)

All decreases in D-dimers, TAT, IL-6, and LDH were sustained in the maintenance phase of treatment. There was no correlation with LDH.

IL=interleukin; LDH=lactate dehydrogenase; TAT=thrombin antithrombin. Data from Weitz IC et al. *Thromb Res.* 2012;130:361-368.

IW This patient's D-dimers came down really nicely. His LDH came down beautifully as well. Unfortunately, his interleukin (IL) 6 level was not assessed; it would have been interesting to observe any changes. One of the least appreciated aspects of PNH and other complement-mediated diseases is the inflammatory component of the disorders and the relationship between the inflammation and the risk of thrombosis. In our clinical study of PNH patients, we specifically looked at plasma markers of thrombin generation (D-dimers and thrombin-antithrombin III-complex [TAT]), and inflammation via IL-6.² We also examined soluble P-selectin, antigenic and functional tissue factor-bearing microparticles (TFMP and fTFMP, respectively), and total plasma microparticle ex vivo factor Xa generation (MPFXa). We enrolled 11 eculizumab-naïve PNH patients who received on-study eculizumab. Blood sampling was performed on day 1, prior to eculizumab treatment, and on days 8, 15, 22, 29, 43, and 90. We observed a statistically significant reduction in D-dimers, TAT, IL-6, soluble P-selectin, and TFMP during the induction phase of treatment, which occurred on days 1–29 (Table 1). This reduction in markers continued during maintenance treatment, which occurred on days 29–90. There was a very strong correlation between the D-dimers, TAT, and IL-6 levels, ie, the markers of hemostatic activation and inflammation. Although serum levels of LDH rapidly decreased, there was no correlation between the reduction in the thrombin generation and inflammation, suggesting that there may be a pathway independent of hemolysis, perhaps inflammatory, inducing thrombosis. Generation of complement protein 5a (C5a) is known to upregulate IL-6 expression and would be inhibited by eculizumab blockade. IL-6 increases monocyte tissue factor expression, which is critical for the generation of thrombosis. We observed a significant reduction in TFMP, but this reduction did not correlate with changes in markers of thrombin generation and inflammation, and ex vivo generation

of MPFXa did not decrease. Ex vivo total microparticle factor Xa generation was inversely correlated with markers of thrombin generation. This suggests that complement activation increases TFMP, and it is reduced by complement inhibition. However, the total microparticles did not change, suggesting that in vivo microparticles per se do not induce thrombosis in PNH. Thus, in vivo thrombin generation in PNH patients appears to occur by a pathway independent of hemolysis and microparticle generation.

Another interesting aspect of this case concerns the “disconnect” between the level of fatigue and the degree of anemia, which is apparent even in clinical trials. For example, the phase III TRIUMPH (Transfusion Reduction Efficacy and Safety Clinical Investigation Using Eculizumab in Paroxysmal Nocturnal Haemoglobinuria) study, a double-blind placebo-controlled trial, examined the safety and efficacy of eculizumab versus placebo in 87 patients.³ Eculizumab was administered by intravenous infusion of 600 mg weekly for the first 4 weeks, followed by 900 mg on week 5, and then 900 mg every 14 days thereafter through week 26. Quality of life was evaluated via the Functional Assessment of Chronic Illness Therapy (FACIT)-Fatigue instrument. The study showed a significant reduction in intravascular hemolysis with eculizumab, as shown by the levels of LDH that were reduced to near normal ($P < .001$) and an associated improvement in fatigue, although the hemoglobin levels lagged (Figure 1). The baseline score of fatigue increased by 6.4 points with eculizumab treatment compared to negative scores of the placebo-treated patients, even though all patients were able to be transfused to a hemoglobin of 10, if needed. Support of this conclusion was also provided by findings from the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ-C30). Notably, however, the anemia did not resolve. Instead, it is the complement activation, possibly through the induction of inflammatory cytokines, such as IL-6, that appears to be the main cause of fatigue in patients with PNH.

Pulmonary hypertension (PHTN) may also contribute to the findings, as it is frequently associated with dyspnea. Dr. Hill's data show the reduction in dyspnea and PHTN that can occur upon treatment with eculizumab. Dr. Hill's study explored the relationship between several markers and measures of PHTN in 73 patients with PNH from the TRIUMPH study.⁴ Forty-seven percent of the patients had levels of N-terminal pro-brain natriuretic peptide (NT-proBNP) of at least 160 pg/mL, which has been shown to indicate PHTN and right ventricular dysfunction. Eighty percent of the patients had reduced right ventricular ejection fraction and 60% had evidence of subclinical PE, yet 50% had no history of transfusions. This again demonstrates the lack of correlation of fatigue to the degree of anemia and transfusion require-

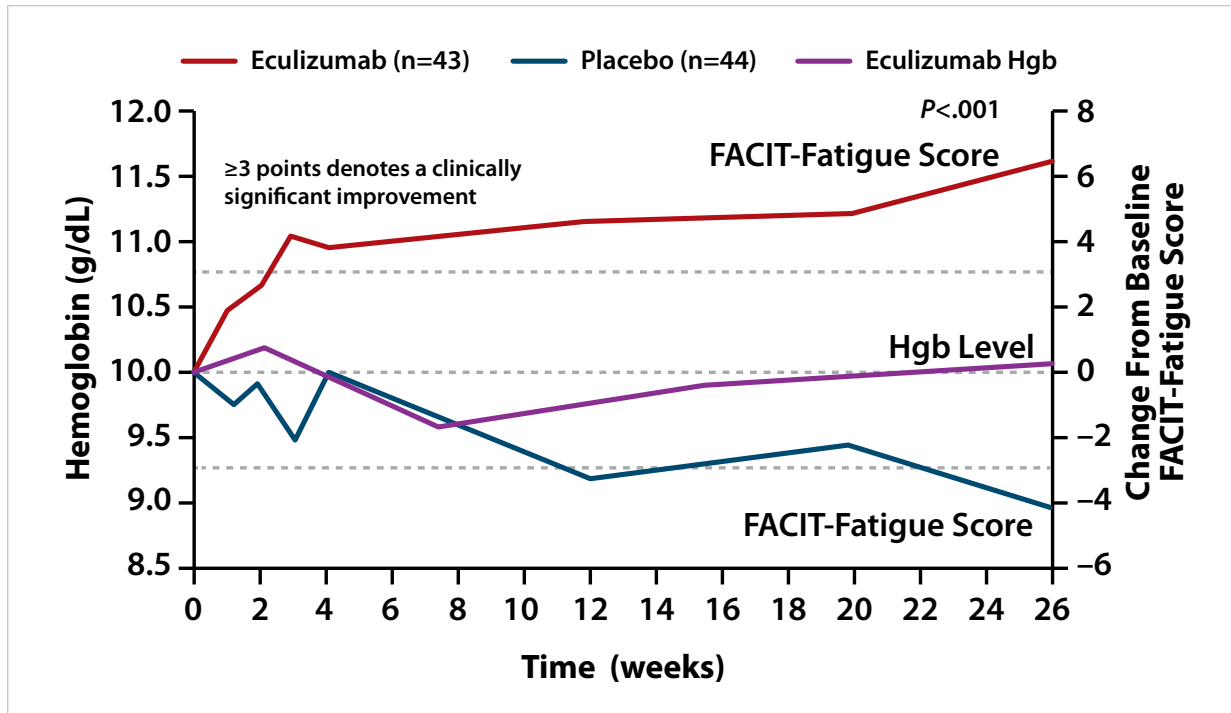


Figure 1. Improvement in fatigue independent of response in the TRIUMPH trial. FACIT=Functional Assessment of Chronic Illness Therapy; Hgb=hemoglobin; TRIUMPH=Transfusion Reduction Efficacy and Safety Clinical Investigation Using Eculizumab in Paroxysmal Nocturnal Haemoglobinuria. Data from Hillmen P et al. *N Engl J Med.* 2006;355:1233-1243. Brodsky R et al. *Blood Rev.* 2008;22:65-74. Hill A et al. *Haematologica.* 2008;93(suppl 1):359; Abstract 0904. Brodsky R et al. *Blood.* 2008;111:1840-1847.

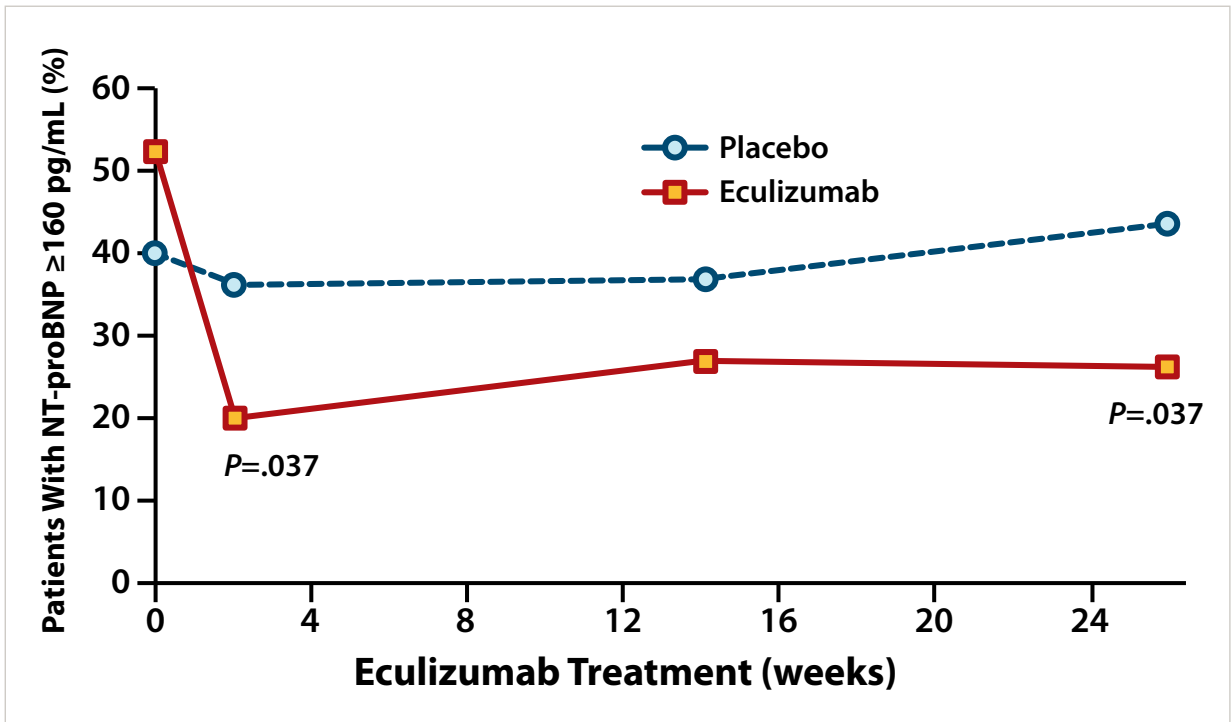


Figure 2. The percent of eculizumab-treated and placebo-treated patients with NT-proBNP levels above 160 pg/mL at 2, 14, and 26 weeks in a subanalysis of the TRIUMPH trial. NT-proBNP=N-terminal pro-brain natriuretic peptide; TRIUMPH=Transfusion Reduction Efficacy and Safety Clinical Investigation Using Eculizumab in Paroxysmal Nocturnal Haemoglobinuria. Adapted from Hill A et al. *Br J Haematol.* 2010;149:414-425.

ment. Eculizumab was administered as described for the TRIUMPH study. At the end of 26 weeks of treatment with eculizumab, the proportion of patients with elevated NT-proBNP was reduced by 50% (Figure 2). In addition, the reduction occurred without significant changes in anemia. The results suggest that monitoring levels of these various markers may provide insights into the thrombotic mechanisms as well.

JS How frequently is this patient being monitored on treatment, and what is being used to monitor him?

IW He is being monitored primarily through his LDH levels. The last time I spoke with him, his LDH was 178 IU/L, his hemoglobin was 13 g/dL, and his D-dimers were less than 110 ng/mL.

JS The response to eculizumab is primarily measured by reduction in the LDH levels, freedom from or a reduction in transfusion requirements, and normalization of D-dimers. Monitoring of flow cytometry is required to ensure that the disease is still there rather than to assess the response. Another important point is the frequency of monitoring. Does the patient undergo formal monitoring every 6 months or every 3 months?

IW Initially, he was monitored every week, then every 2 weeks starting on day 29, until he reached day 90 and showed stability. Currently, he is being monitored every 3 months.

JS How often does he undergo flow cytometry?

IW Flow cytometry was not repeated. It is performed once a year.

Anita Hill, MBChB (Hons), MRCP, FRCPath, PhD
Our regular monitoring for all patients with PNH, particularly those on eculizumab, is every 3 months. We check for transfusion history and blood count, and assess end organ function. All patients on therapy undergo assessment of PNH clone size every 3 months.

IW PNH clone size is checked every 3 months?

AH Yes. It could be argued that less frequent monitoring might be appropriate for some of the patients who have been on therapy for 10 years and have remained with very stable percentages of PNH cells. We have seen a small number of patients who have a clone size that is beginning to fall. We have 1 patient who will likely stop treatment because of falling clone size in the next 18 months, and a further 2 who have been able to stop therapy due to spontaneous resolution of their PNH clone. This monitoring informs

us of how the bone marrow might be impacting the clone. Similarly, the percentage of PNH cells may also increase. This is a further indication for regular monitoring of both the proportion of PNH cells and end-organ damage.

JS That was the point that I was trying to highlight. At my institution, patients are monitored at least once every 6 months. The International Clinical Cytometry Society (ICCS) has defined the appropriate testing for the diagnosis and monitoring of PNH. In patients with established and stable disease, it was recommended that the clone size be reassessed at annual intervals unless changes in clinical or hematologic parameters occur, in which case more frequent monitoring is required.⁵

IW We have trouble getting such tests done repeatedly because they are not performed in-house. In this patient, for example, his insurance required that flow cytometry be performed at his primary site. Such constraints make it difficult for us to increase the frequency of these tests. If we are able to get it done, we try to repeat the flow cytometry yearly. If there were a consensus statement from either the PNH Interest Group or the ICCS for more frequent testing, it would be easier to do it. Currently, the recommendations from the ICCS are for yearly testing.

JS It is not completely uncommon for a patient to have a change in the underlying disease status. I had one patient whose clone disappeared. This particular patient was having problems with fatigue, transfusion dependence, and quality of life in general, rather than end-organ damage.

Acknowledgment

Dr. Weitz is a consultant and advisor for and has received honoraria and research funding from Alexion Pharmaceuticals. Dr. Szer is a member of the Speakers Panel and has received honoraria for talks and submissions from Alexion Pharmaceuticals. Dr. Hill has served as a consultant and speaker for Alexion Pharmaceuticals, Inc.

References

1. Weitz I, Meyers G, Lamy T, et al. Cross-sectional validation study of patient-reported outcomes in patients with paroxysmal nocturnal haemoglobinuria. *Intern Med J*. 2012 Aug 22. doi: 10.1111/j.1445-5994.2012.02924.x. [Epub ahead of print]
2. Weitz IC, Razavi P, Rochanda L, et al. Eculizumab therapy results in rapid and sustained decreases in markers of thrombin generation and inflammation in patients with PNH independent of its effects on hemolysis and microparticle formation. *Thromb Res*. 2012;130:361-368.
3. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2006;355:1233-1243.
4. Hill A, Rother RP, Wang X, et al. Effect of eculizumab on haemolysis-associated nitric oxide depletion, dyspnoea, and measures of pulmonary hypertension in patients with paroxysmal nocturnal haemoglobinuria. *Br J Haematol*. 2010;149:414-425.
5. Borowitz MJ, Craig FE, DiGiuseppe JA et al. Guidelines for the Diagnosis and Monitoring of Paroxysmal Nocturnal Hemoglobinuria and Related Disorders by Flow Cytometry. *Cytometry Part B (Clinical Cytometry)* 2010;78B:211-230.

Paroxysmal Nocturnal Hemoglobinuria: Case 2

Anita Hill, MBChB (Hons), MRCP, FRCPath, PhD
Consultant Haematologist and Honorary Senior Lecturer
Department of Haematology
St James' Institute of Oncology
Leeds Teaching Hospitals
Leeds, United Kingdom

The patient was a 19-year-old university student with no medical history. His university degree was being funded by the Royal Air Force. During one of his Air Force training sessions, he became quite exhausted. He went back home and passed urine the color of Coca-Cola. He attended the Emergency Department and was found to have a hemoglobin of 7 g/dL. He first underwent a computed tomography (CT) scan, which excluded genitourinary problems. He was then transfused with 2 units of packed red cells. A junior doctor diagnosed him with march hemoglobinuria. The patient was told that he would get better with some rest.

So, as many university students tend to do when they do not feel well, he went back home to his parents. He did not feel better, and he was admitted to his local hospital. He was continuing to pass dark urine. They noted on admission that his creatinine was 260 $\mu\text{mol/L}$, with an LDH of over 5,000 U/L. The local doctors quickly made the diagnosis of PNH. They excluded renal vein thrombosis with a renal ultrasound scan as a cause of his acute renal impairment. They gave him intravenous fluid, which did actually start to improve his creatinine, commenced him on prophylactic anticoagulation, gave him folic acid, and then sent him to St. James' University Hospital in Leeds for a review.

When we saw him, we thought that either an infection or strenuous activity during the training session had precipitated this attack. He had previously been extremely fit and well. His hemoglobin, close to transfusion, was 9.6 g/dL; he had a normal white cell count, a normal neutrophil count, and a normal platelet count of 260,000/ μL . Creatinine had improved to 149 $\mu\text{mol/L}$. LDH was still significantly raised, and flow cytometry showed 87% PNH granulocyte with 22% red blood cells. The discrepancy between the granulocyte and red blood cell clone is found because hemolysis reduces the red cell clone size, and blood transfusion reduces the proportion of PNH red cells.

We then had a long discussion with him about his disease, and what it might mean to him. Obviously, we discussed commencing eculizumab therapy. His diagnosis was a classical, highly hemolytic PNH. He had already needed one transfusion, and he had experienced renal impairment. Although this impairment was probably reversible, it showed that he was clearly at risk of continuing renal damage.

Understandably for a 19-year-old, he was concerned about the potential impact of having 2 weekly infusions for the rest of his life. He was also concerned about staying on anticoagulation, which could be difficult given his responsibilities in the Royal Air Force. He said that he was "fine" and would see how he got on. Although he was more tired than usual, he was coping with his usual exercise regimen. We agreed to follow him intermittently. When he came back to visit us 5 weeks later, he said he was managing once again. His hemoglobin was still about 10 g/dL. His LDH was still well above 2,500 U/L. He did also admit that the Royal Air Force was not putting him on any strenuous activity.

With continuing follow-up, he reported erectile dysfunction, which he discussed with his primary care physician. His hemoglobin again fell to 7.5 g/dL. He started taking iron. He kept reporting that he felt well enough to manage, and we carried on discussing eculizumab. His renal function actually improved back to normal levels. We noticed some proteinuria on dipstick testing. We routinely evaluate for proteinuria in all patients, particularly those who are untreated, to look for early signs of tubular damage. His LDH was consistently between 2,500 and 5,000 U/L. At university, his examination results started to fall by 20%, and, unlike most university students, he was now going to bed at 9 PM. We measured his pulmonary pressures 1 year after presentation, and his central pulmonary artery pressure was 47 mm Hg. At this point, we told him that he was now not just developing symptoms, but also renal consequences and pulmonary consequences of chronic

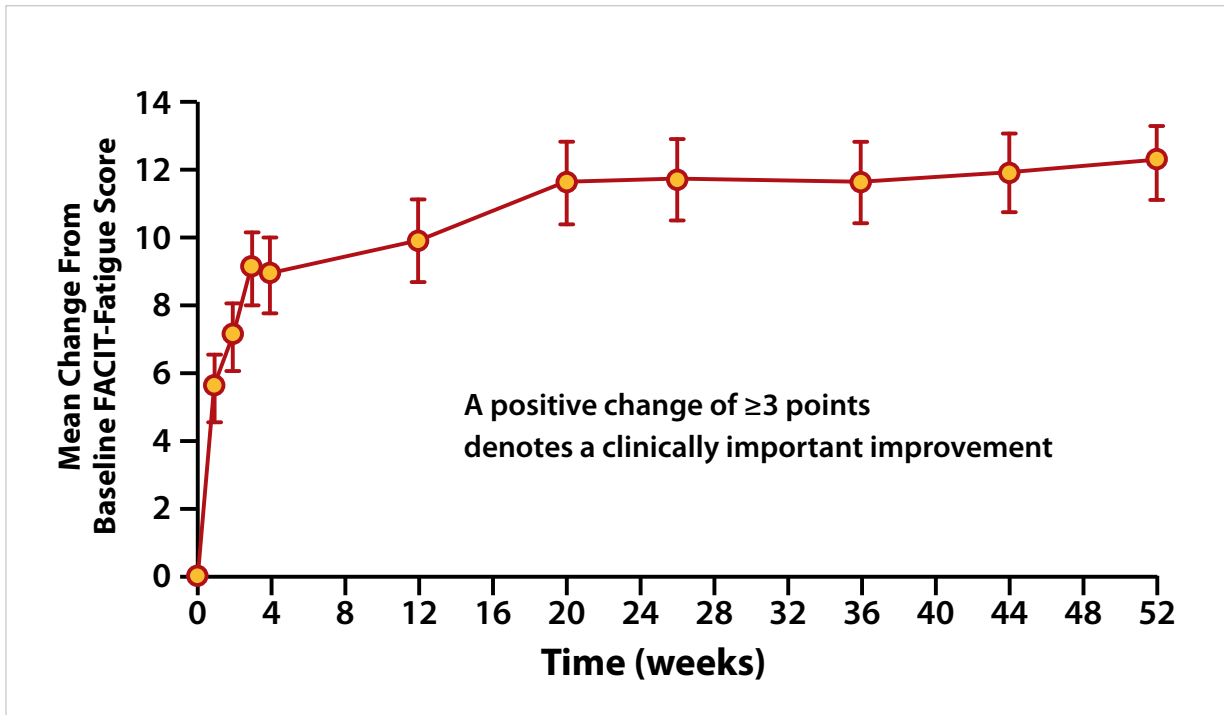


Figure 3. Improvement of fatigue in the SHEPHERD trial. FACIT-F=Functional Assessment of Chronic Illness Therapy-Fatigue instrument. $P < .001$ at all measured time points as compared with baseline using the signed rank test. SHEPHERD=Safety in Hemolytic PNH Patients Treated With Eculizumab. Adapted from Brodsky RA et al. *Blood*. 2008;111:1840-1847 and Brodsky RA et al. *Blood*. 2008;111:1840-1847.

hemolysis. We stressed that these conditions were having an impact on his life and potentially his future with the deterioration of the examination results.

After consideration, he started therapy in September 2011. The impact on his life was huge. His energy level returned to normal, and he had forgotten what that had felt like. In January 2012, we repeated his central pulmonary artery pressure, which was now normalized. Luckily, his disease was caught in time to reverse some of these consequences. His latest red cell clone size shows that PNH granulocyte is 98.5%, with total red cells of 78%.

Discussion

Jeff Szer, MBBS, FRACP Did the patient require transfusion along the way?

AH He required just 1 transfusion at presentation, and a further one later during an infection. This case is a good example of a patient who, despite a high level of hemolysis, required very infrequent red cell transfusions. This is not at all uncommon and is accounted for by the compensatory increased erythropoiesis allowing the maintenance of reasonable hemoglobin levels. It should be understood that these patients are still at risk of all the complications of PNH.

JS Would he have been eligible for enrollment in the SHEPHERD (Safety in Hemolytic PNH Patients Treated With Eculizumab) study? This open-label, non-placebo-controlled, phase III trial enrolled 97 patients at 33 international sites.¹ Eculizumab was administered as it was for the TRIUMPH study, but treatment lasted for 52 weeks. SHEPHERD showed an 87% reduction in hemolysis, as measured by LDH levels ($P < .001$). The FACIT-Fatigue Instrument showed that fatigue scores improved by 12.2 \pm 1.1 points from baseline ($P < .001$), bearing in mind that an increase of at least 3 points denotes a clinically important improvement (Figure 3). This result is consistent with the TRIUMPH findings that also showed a clinically important improvement in fatigue from eculizumab based on the same questionnaire ($P < .001$).¹⁻³

AH Yes, he would have been eligible for the previous SHEPHERD trial that highlighted how patients suffer due to the hemolysis, which is not always represented by their transfusion requirements.

Ilene Ceil Weitz, MD An important point is that even though the patient's hemoglobin was approximately 10 g/dL, he still had the symptomatology consistent with PNH. So, again, we have this disconnect: the patient received a transfusion, but he did not feel better

Table 2. Pulmonary Hypertension in PNH

<p>Three different patient cohorts show high prevalence of pulmonary hypertension using 3 different measurements:</p> <ul style="list-style-type: none"> • 47% of patients with elevated NT-proBNP ≥ 160 pg/mL¹ • 43% of patients with mild to moderate and 7% severe pulmonary hypertension as evidenced by cardiac Doppler imaging² • 100% of patients with elevated BNP³ <ul style="list-style-type: none"> – 80% with reduced right ventricular ejection fraction – 60% had evidence of subclinical PE – 50% had no history of transfusions

NT-proBNP=N-terminal pro-brain natriuretic peptide; PE=pulmonary embolism; Data from 1. Hill A et al. *Blood*. 2008;112(11): Abstract 486. 2. Hill et al. *Br J Haematol*. 2006;133(suppl 1): Abstract 316. 3. Hill et al. *Blood*. 2006;108(11): Abstract 979.

(Figure 3). So it was not the hemoglobin level that was the best indicator of disease, but the fact that he had ongoing complications, such as hemoglobinuria and PHTN (Table 2). It is the complement inhibition brought about by treatment with eculizumab, and not the transfusion, that changed the outcome for him.

AH Yes. We have an increasing understanding about the symptoms seen in PNH, with nitric oxide depletion likely to play a significant role. The fatigue is often out of proportion to the hemoglobin level. Anemia is therefore not the only contributor to this symptom in this disease. Nitric oxide depletion and the presence of a chronic inflammatory state due to the release of C5a also significantly contribute to fatigue.

JS He probably would have had an unnecessary cystoscopy if his urine had been red rather than black.

AH Absolutely. These patients, probably not surprisingly, are sometimes referred to a urologist and undergo investigations for a long period before (hopefully) someone realizes they do not have hematuria but in fact hemoglobinuria.

JS It is surprising to see how many patients with hemoglobinuria undergo a couple of cystoscopies before a blood count is performed.

IW I have one patient who had undergone 5 cystoscopies, starting when she was in her 30s. She had been diagnosed with possible bladder cancer but *never* had positive cytology, and spent 15 years with hemoglobinuria before she was diagnosed with PNH.

Acknowledgment

Dr. Hill has served as a consultant and speaker for Alexion Pharmaceuticals, Inc. Dr. Szer is a member of the Speakers Panel and has received honoraria for talks and submissions from Alexion Pharmaceuticals. Dr. Weitz is a consultant and advisor for and has received honoraria and research funding from Alexion Pharmaceuticals.

References

1. Brodsky RA, Young NS, Antonioli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2008;111:1840-1847.
2. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2006;355:1233-1243.
3. Cella D, Eton DT, Lai JS, Peterman AH, Merkel DE. Combining anchor and distribution-based methods to derive minimal clinically important differences on the Functional Assessment of Cancer Therapy (FACT) anemia and fatigue scales. *J Pain Symptom Manage*. 2002;24:547-561.

Paroxysmal Nocturnal Hemoglobinuria: Case 3

Jeff Szer, MBBS, FRACP
Professor/Director
Department of Clinical Haematology & BMT Service
Royal Melbourne Hospital
Melbourne, Australia

The patient was, at the time of diagnosis, a 44-year-old dairy farmer. She presented with a painful deep venous thrombosis and had an unsuspected PE at the time of diagnosis. She was anticoagulated and at the time had a hemoglobin of 6.7 g/dL. She received a blood transfusion. Over the next couple of years, she received an average rate of about 1 unit of red blood cells per month. Subsequently, a bone marrow biopsy showed erythroid hyperplasia, which prompted flow cytometry. A diagnosis of PNH was made, with a red cell clone of 30% and a granulocyte clone of 52%. The patient's LDH level was 4,890 U/L, which is about 10 times the upper limit of normal. She continued to receive transfusions and began anticoagulation therapy with warfarin, when she enrolled in the TRIUMPH trial.¹

TRIUMPH randomized 87 patients to receive either eculizumab, administered as described previously, or placebo for a total of 26 weeks. Of the 43 patients randomized to eculizumab, 21 (49%) showed stabilization of hemoglobin levels in the absence of transfusions ($P < .001$). Patients on placebo received a median 10 units of packed red blood cells versus 0 units for patients in the treatment arm ($P < .001$). A reduction in hemolysis, as determined from LDH analysis, was also observed in patients treated with the antibody ($P < .001$). As mentioned above, quality of life improved with eculizumab treatment as measured by the FACIT-Fatigue Instrument ($P < .001$). Serious adverse events were recorded for 4 patients in the drug treatment arm and 9 patients in the placebo arm, and all patients recovered without sequelae.¹

The patient described in this case study was enrolled in the TRIUMPH trial in 2005 and showed a rapid improvement with eculizumab treatment. She continued on anticoagulation as the trial required and had a marked reduction in transfusion dependence. She required roughly 1 transfusion per year over the next couple of years. Transfusions were usually precipitated by a respiratory infection or especially busy times in the dairy.

In late 2008, she wanted to stop anticoagulation. She had been on it for 5 years, without a further event, and asked me about data supporting this approach.

I presented what information I had, although it was mainly regarding primary prophylaxis. She made the decision to discontinue anticoagulation therapy.

In mid 2009, she had a cycling accident and fell over. She developed a painful leg the next day. She was diagnosed with a deep venous thrombosis (DVT) in the same leg as her pretreatment DVT, and a pulmonary embolus. She was re-anticoagulated and stayed on that therapy while continuing to receive eculizumab. Her level of LDH was maintained just at the upper limit of normal; the last result was approximately 460 U/L. The granulocyte clone size is now 98%, and the red cell clone size is 4%.

The major impact on her life has been the ability to wake up at 3:30 AM to milk the cows, and then make it through an entire day. She reacquired a social life that she had lost but had not quite realized why.

Discussion

JS This case raises the question of whether secondary prophylaxis should be administered with anticoagulation. I know that at Leeds, your policy is to continue anticoagulation in patients who had previously had a thrombosis. Is that correct?

Anita Hill, MBChB (Hons), MRCP, FRCPath, PhD That is our standard. We have stopped secondary anticoagulation now in 3 patients: 2 because of varices and 1 because of a subdural hematoma. We probably are being overly cautious about continuing secondary anticoagulation, knowing the considerable and significant reductions in thrombosis for patients with PNH on eculizumab therapy. However, we are certainly stopping it when we are concerned about bleeding risk.

Ilene Ceil Weitz, MD I think that is a question that the PNH registry should answer because there will never be a trial. Among patients who are receiving primary prophylaxis only, we just follow their D-dimers. But in patients who have conditions such as Budd-Chiari syndrome or major pulmo-

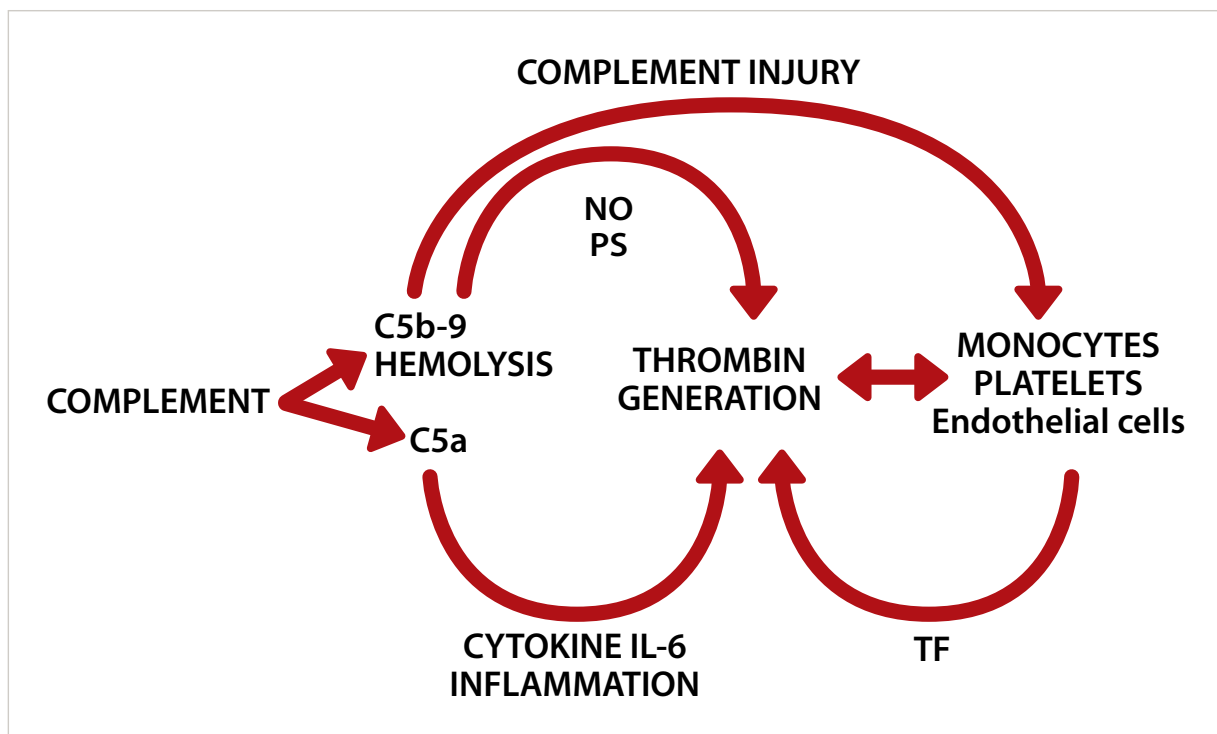


Figure 4. Many mechanisms of thrombosis have been proposed, and many of them lead back to platelet activation. IL=interleukin; NO=nitrous oxide; PS=phosphatidylserine; TF=tissue factor.

nary emboli, the question is, can anticoagulation be stopped once they are on treatment? Here is a patient who was on treatment but had another event, but it was a provoked event presumably. Right?

JS Yes. It is the only case of post-treatment, provoked thrombosis that I have seen.

IW I have not seen any such cases, and we have stopped anticoagulation in several patients.

AH Likewise, we also have not had any cases of thrombosis in patients in whom we have stopped anticoagulation after the initiation of eculizumab therapy.

JS Is there a consensus about continued treatment in someone without comorbidities that might suggest an increased bleeding risk, such as esophageal varices or a history of cerebral events? In this case, should she have stopped the secondary prophylaxis? Would you have stopped her secondary prophylaxis in Leeds?

AH As mentioned, we would stop secondary anticoagulation if we had concerns about bleeding risk. We have already seen very significant hemorrhages in this group. In other patients, we still believe that it is too early to

make that decision. However, among those patients in whom we have stopped secondary prophylaxis, none have experienced a recurrent thrombosis.

JS In meetings that I speak at, I quite frequently hear the question, “Why not give the patient antiplatelet agents?”

AH The answer I give to that question is that many mechanisms of thrombosis have been proposed, and many of them lead back to platelet activation (Figure 4). However, complement activation of the platelets would still occur even if the cyclooxygenase pathway is blocked. There is no benefit in these patients for the use of antiplatelet agents to prevent thrombosis related to PNH.

IW Thromboses in these patients mostly occur on the venous side, and the use of aspirin for secondary prophylaxis from the venous side is pretty poor. There is, however, one paper that has suggested a benefit from aspirin. A multicenter, double-blind trial suggested that aspirin reduced the risk of recurrence of unprovoked venous thromboembolism without an increase in the risk of bleeding,² but this study was not performed in PNH patients.

AH The issue with PNH is that obviously quite a significant proportion of the thromboses that occur are not venous.

This means that thrombosis in PNH comes back to platelet activation. In the past, we investigated for PNH more in patients who had Budd-Chiari syndrome and cerebral venous thromboses, and less in patients who presented with arterial strokes and myocardial infarctions. Whereas now, when we are looking at young patients with unexplained strokes, we are finding PNH. We have had several young people with strokes this last year diagnosed with PNH.

JS Yes. So the bottom line is that we have no data to support the use of antiplatelet agents.

AH Absolutely.

IW For this patient, it is important to stress that her thromboembolic event was not spontaneous, it was provoked. She did not stop her eculizumab. Is that correct?

JS Not for a minute.

IW So it is a provoked event due to the trauma of the bicycle accident.

AH Was she adequately dosed?

JS Her LDH level did not budge. She still required the occasional transfusion in the context of infections or a busy milking period. The only provoking event was the cycling accident, which caused significant leg trauma. That could have happened to anyone. However, she is not going to ask to stop anticoagulation again.

Acknowledgment

Dr. Szer is a member of the Speakers Panel and has received honoraria for talks and submissions from Alexion Pharmaceuticals. Dr. Hill has served as a consultant and speaker for Alexion Pharmaceuticals, Inc. Dr. Weitz is a consultant and advisor for and has received honoraria and research funding from Alexion Pharmaceuticals.

References

1. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2006;355:1233-1243.
2. Becattini C, Agnelli G, Schenone A, et al. Aspirin for preventing the recurrence of venous thromboembolism. *N Engl J Med.* 2012;366:1959-1967.

Slide Library

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- A rare, acquired disorder characterized by chronic intravascular hemolysis as the primary clinical manifestation
- Morbidities include anemia, thrombosis, and bone marrow failure
- Caused by the acquisition of a somatic mutation of the gene phosphatidylinositol glycan anchor (PIG-A) in a multipotent hematopoietic stem cell (HSC), with clonal expansion of the mutated HSC. The mutation causes a deficiency in the synthesis of glycosylphosphatidylinositol (GPI)
- Although all blood cells are affected—and are therefore susceptible to complement-mediated attack—lysis of red blood cells is the most obvious manifestation of the disorder

Impaired Quality of Life in PNH

- Burden of fatigue and impaired quality of life was extensive in a study of 29 PNH patients:
 - 96% complained of fatigue
 - 76% were forced to modify their daily activities to manage their PNH
 - 17% were unemployed due to PNH
 - >50% reported abdominal pain, headache, and dyspnea
 - Patients commonly reported dysphagia (41%) and erectile dysfunction (100% in males)
 - The symptoms were not related to anemia or transfusion. Most symptoms were rated as moderate to very severe, and a substantial majority reported distress associated with the symptoms

Data from Weitz I et al. *Intern Med*. 2012 Aug 14. doi: 10.1111/j.1445-5996.2012.03914.x. [Epub ahead of print]

Eculizumab in PNH

- A humanized monoclonal antibody against the C5 complement protein
- The only effective drug therapy for PNH
- Prevents cleavage of the C5 protein by C5 convertase, in turn preventing generation of C5b-9 and resultant hemolysis of cells lacking the CD59 surface protein

The TRIUMPH Trial

- Examined the safety and efficacy of eculizumab versus placebo in 87 patients
- Eculizumab was administered by intravenous infusion of 600 mg weekly for the first 4 weeks, followed by 900 mg on week 5, and then 900 mg every 14 days thereafter through week 26
- The study showed a significant reduction in intravascular hemolysis with eculizumab
- The baseline score of fatigue increased by 6.4 points with eculizumab treatment
- The anemia did not completely resolve. Instead, it was the hemolysis that appeared to be the main cause of diminished quality of life in patients with PNH

TRILIMPA—Transfusion Reduction Efficacy and Safety Clinical Investigation Using Eculizumab in Paroxysmal Nocturnal Hemoglobinuria.
Data from Hillmen P et al. *N Engl J Med*. 2006;355:1233-1243.

A Subanalysis of the TRIUMPH Trial

- Explored the relationship between several markers and measures of pulmonary hypertension in 73 patients with PNH from the TRIUMPH study
- 47% of patients had levels of N-terminal pro-brain natriuretic peptide of at least 160 pg/mL, which has been shown to indicate pulmonary hypertension
- 80% of patients had reduced right ventricular ejection fraction, 60% had evidence of subclinical PE, and 50% had no history of transfusions
- Within 2 weeks of treatment with eculizumab, the proportion of patients with elevated NT-proBNP was reduced by 50%. The reduction occurred without significant changes in anemia
- The results suggest that monitoring levels of these various markers may provide insights into the thrombotic mechanisms

Hill A et al. *Br J Haematol*. 2010;142:414-419.

The SHEPHERD Trial

- Open-label, non-placebo-controlled, phase III trial that enrolled 97 patients at 33 international sites
- Eculizumab was administered as it was for the TRIUMPH study, but treatment lasted for 52 weeks
- There was an 87% reduction in hemolysis, as measured by LDH levels ($P < .001$)
- The FACIT—Fatigue Instrument showed that fatigue scores improved by 12.2 +/- 1.1 points from baseline ($P < .001$). (An increase of at least 3 points denotes a clinically important improvement.)
- This result is consistent with the TRIUMPH findings that also showed a clinically important improvement in fatigue from eculizumab based on the same questionnaire ($P < .001$)

FACIT—Functional Assessment of Chronic Illness Therapy; SHEPHERD—Safety in Hemolytic PNH Patients Treated With Eculizumab.
Data from Brodsky RA et al. *Blood*. 2008;111:1840-1847.

Monitoring of Patients Receiving Treatment for PNH

- Transfusion history
- Blood count
- Assessment of end-organ function
- Assessment of PNH clone size

Thrombosis in PNH

- Rates of clinical thrombotic events in recent trials
 - TRIUMPH: venous 85% and arterial 15%¹
 - AEGIS: 18%²
 - South Korean Registry: 18%³
- Leading cause of death¹
 - Accounts for 40–67% of deaths¹
 - First thrombotic event can be fatal
 - Median time to TE is 2.1–2.3 years from diagnosis⁴
 - First TE increases risk of death 5-fold to 10-fold¹
 - Contributes to end-organ damage

TE=thromboembolism.
Data from 1. Hillmen P et al. *Blood*. 2012;119:1217-1228. 2. Kimbura Y et al. *Int J Hematol*. 2012;93:46-51. Lee JH et al. *Hematologica*. 2012;97(1):Abstract 955 and 956. 3. Park JH et al. *Blood*. 2008;112:327-336.

Risk of Thrombosis in PNH

- Risk of thrombosis is inherent with PNH and is difficult to measure
 - Rough correlation with higher clone size, but there are multiple reports of serious thrombotic events in patients with smaller clone size (as low as 9%)
 - No correlation with transfusion dependency, the degree of anemia, or the degree of hemolysis
 - Thrombosis is reported in patients with low levels of hemolysis or no hemolysis (PNH II cells, PIG-M mutations)

PIG=phosphatidylinositol glycan.

Why Antiplatelet Agents Are Not Used in PNH

- Many mechanisms of thrombosis have been proposed, and many of them lead back to platelet activation. However, complement activation of the platelets would still occur even if the cyclooxygenase pathway is blocked
- Thromboses in PNH patients frequently occur on the venous side, and the use of aspirin for secondary prophylaxis of thromboses from the venous side has poor results
- There is no benefit for the use of antiplatelet agents to prevent thrombosis in patients with PNH

For a free electronic download of these slides, please direct your browser to the following web address:
http://www.clinicaladvances.com/index.php/our_publications/hem_onc-issue/ho_november_2012/

