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Information for Authors:

Purpose

The mission of the *University of Virginia Journal of Medicine* is to provide residents, fellows, and faculty members the opportunity to publish original materials generated from their experiences in patient care or patient care–related research. **The journal will give housestaff at the University of Virginia Health System the opportunity to work with the faculty in writing medical case reports, thus providing a forum for learning about the process of journal article submission and revision.** In addition, the journal offers referring physicians in the state of Virginia, alumni of the medicine training programs, and healthcare providers associated with the Universtiy of Virginia Health System the opportunity to learn from the breadth of clinically based educational experiences generated from patient care at the University of Virginia Health System.

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Only original, unpublished materials will be considered for publication. Submissions should be made electronically to Joy Hilton (njh3s@hscmail.mcc.virginia.edu). When submitting a manuscript, authors should provide full disclosure of any duplicate publication of any content of the paper in a cover letter to the Editor. Funding sources and any potential conflicts of interest reported in the cover letter should also be included in the manuscript text file.

Manuscript Format

- Authors are encouraged to follow the AMA writing style as outlined in *Writing and Publishing in Medicine,* 3rd Edition, Edward J. Huth, MD. Accepted manuscripts are edited in accordance with the *American Medical Association Manual of Style: A Guide for Authors and Editors,* 9th edition. Authors are also encouraged to refer to this guide when preparing manuscripts for submission.

- All submissions should include a title page, text, references, and as appropriate, figure legends, tables, and figures. For preparation of accepted manuscripts, a manuscript text file in Word must be submitted. Figure images should be submitted in separate .jpg files, with labels. Legends for all figures should be included at the end of the manuscript text file. Tables must be submitted as text, not images, and included in the manuscript text file.

- Abbreviations should not be used in the title or abstract, even commonly used abbreviations. Limit the use of abbreviation in the text, and expand all abbreviations at first mention in the text. All measurements should be expressed in SI units. Generic drug names are preferred.

- The manuscripts must be free of any identifying patient information in order to respect confidentiality.

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  1. Herbert West, MD, Assistant Professor of Medicine, Division of Reanimation, Miskatonic University, Arkham, MA

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Abstracts

A structured abstract of no more than 300 words for reports of original data. Structured abstracts should include the following sections: Background, explaining the clinical (or other) importance of the study and stating the objective or question addressed. Methods, describing the basic design of

the study, patient or participant characteristics, and interventions. Results, reporting and quantifying the main outcomes of the study. Conclusions, providing conclusions of the study supported by the results, along with implications for clinical practice.

- For other manuscripts, include an unstructured abstract of no more than 200 words that summarizes the objective, main points, and conclusions of the article. No information should be reported in the abstract that does not also appear in the text of the manuscript.

References

- All information not considered statements of common knowledge must be supported by citation of published articles in the medical scientific literature.

- Literature sources must be cited according to AMA guidelines. List all authors and/or editors up to 6; if more than 6, list the first 3 followed by "et al." Number references in the order they appear in the text; do not alphabetize. In text, tables, and legends, identify references with superscript Arabic numerals. Abbreviate names of journals according to PubMed guidelines.

Examples of Reference Style:

Journal Article:

1. Crusher BC, McCoy LH, Bashir JS, et al. Extraterrestrial transfusion methods. *J Interplanetary Med.* 2800;13:53-65.

Book:

2. West H. *Reanimation in Theory and Practice.* Arkham, MA: Miskatonic University Press; 1923.

Guidelines for Article Review Process

Manuscripts will be blindly reviewed by two members of the review board. Decisions regarding acceptance for publication will be based on the strength of the paper compared with other papers in the literature, the need for the *University of Virginia Journal of Medicine* to represent a balanced picture of important advances in internal medicine, and the number of accepted papers in the paper's category and topic area. In addition, reviewers will score submissions based on the following criteria.

i. Originality of case presentation
ii. Clarity of teaching points
iii. Balanced and evidence-based representation of recommendations
iv. Quality of the writing

UVa Journal Article Categories:

Clinical Vignettes: *length - 800-1600 words*

- Clinical vignettes describe patients with classic presentations of rare diseases or common diseases with unusual or interesting aspects. Authors are encouraged to present a brief review of pertinent literature and discuss salient parts of the patient diagnosis. Clinical Vignettes are coauthored by the resident or fellow and the attending physician who supervised the care of the patient and focus on one or two teaching points related to diagnosis, management, or treatment.

UVa Images in Medicine: *length - maximum 250 words*

- Presentation of a radiographic image or digital photograph of an intriguing patient case accompanied by a brief case report. Authors should focus on the diagnosis and management of underlying pathophysiology related to the presented image and associated medical condition.

Medical Grand Rounds: *length - 1600-3200 words*

- A review article written by an attending physician who recently presented at Medical Grand Rounds at the University of Virginia. Specifically, this article should provide readers a thorough overview of recent scientific and technologic advances, discussed during the Grand Rounds and developed at the University of Virginia, which have contributed to the overall understanding and management of specific conditions.

Clinical Review Article: *length - 1600-3200 words*

- A comprehensive review article written by an attending physician based on a thorough assessment of the literature with the goal of outlining the current understanding of the pathophysiology and up-to-date practice guidelines for specific clinical topics.

Clinical Commentary: *length - 1600-3200 words*

- The Clinical Commentary offers attending or resident physicians an opportunity to provide a unique clinical perspective on a component of patient care, education, or medical advancements. The scope of this submission is quite broad and may incorporate discussion of controversial issues in the practice of medicine, topics related to or examples of patient care that have affected the author's personal or professional outlook, and/or commentaries about health care policy or public health.

The Academic Hospitalist Corner: *length - 1600-3200 words*

- This section is dedicated to the emerging field of hospital medicine. Article submissions may be case reports, clinical reviews, perspective pieces, and/or commentaries on medical education and training as related to hospitalist medicine.

Chief Resident Clinical Medicine Conference: *length - 1600-3200 words*

- A report that describes a rare or unusual case, with an emphasis on processes of differential diagnosis, work-up, and management that are also applicable to commonly encountered medical conditions. The article should include a thorough discussion of several diagnostic dilemmas with the goal of addressing both the management of complex pathophysiology and the process of systematically arriving at difficult diagnoses.

Tutorials in Medicine: *length - 1600-3200 words*

- A classic presentation of a disease process with board-style questions about the case. This is followed by a discussion of one or more of the following: differential diagnosis, pathophysiology, management, or treatment. These should be authored by an attending physician with resident or fellow physician collaboration.

Clinical Research : *length - 1600-3200 words*

- Presentation of original data from clinical research conducted wholly or in part at the University of Virginia. Research accepted for publication must be current, well-executed, and applicable to patient care.

Kaposi Sarcoma

Charles Malpass, MD, Fellow Physician, Department of Medicine, Division of Pulmonary and Critical Care Medicine
Eugene Barrett, MD, PhD, Professor of Medicine and Pediatrics, Division of Endocrinology and Metabolism

CASE REPORT

An 86-year-old Hispanic man with no previous medical history presented to the Emergency Department of the University of Virginia Health System because of swelling and pain of his right leg that had started more than 5 years previously. Although the size had fluctuated over time, the leg had progressively grown larger, leading to immobility. In addition, superficial dark nodular areas had developed 6 months prior to presentation. These areas were painful and periodically over time would enlarge and then regress. The patient denied systemic symptoms including fever, chills, fatigue, and night sweats, and had no known sick social contacts. He was born and resided in the outskirts of Mexico City prior to moving to the United States 15 years earlier.

Photographs of Presenting Symptoms

Results of the patient's physical examination are illustrated by the photographs in Figure 1, which show severe unilateral lymphedema of the right leg with a distribution of dark brown, firm nodules and plaques around the knee.

Laboratory Testing

Results of a comprehensive metabolic panel, complete blood count with differential, protime, and prothrombin time were normal. An enzyme-linked immunoassay test for HIV was negative. Attempts to aspirate samples with 22- and 16-gauge needles were unsuccessful.

Radiology

Doppler ultrasound of the right lower extremity showed no evidence of venous occlusion.

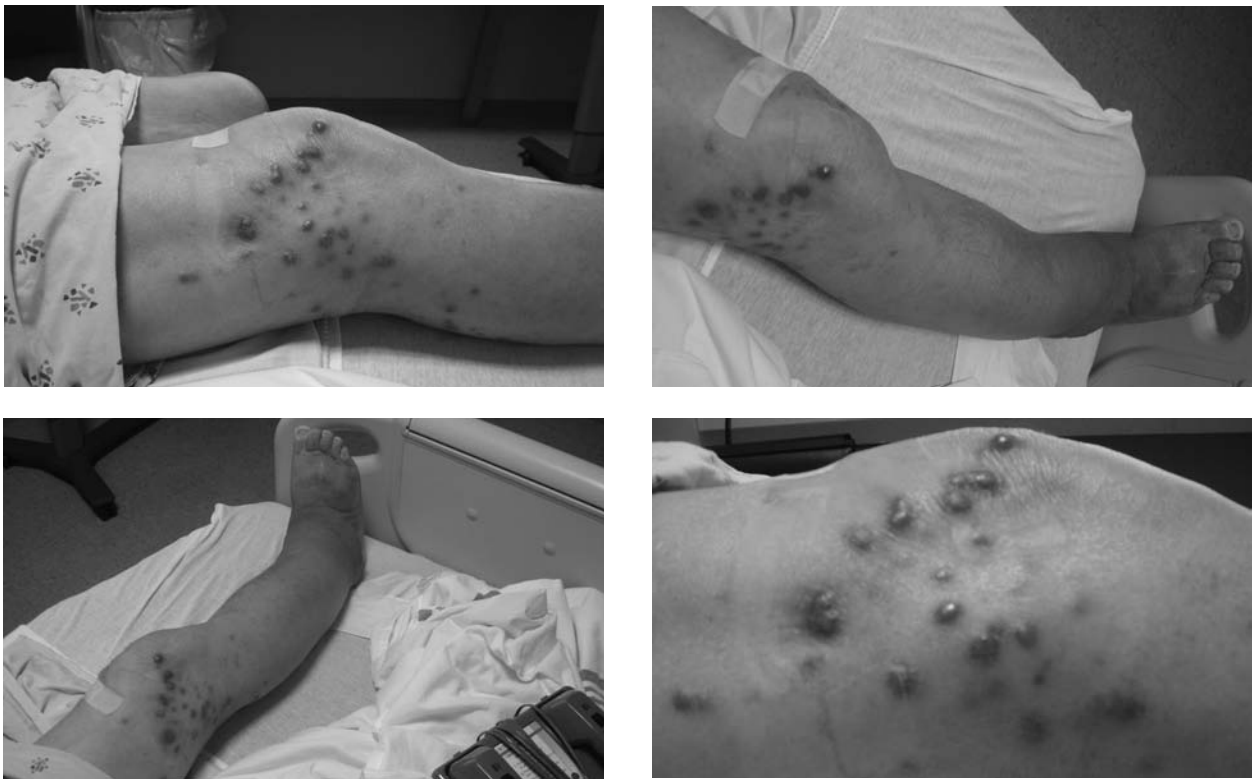

Figure 1. Photographs of the patient's right leg. The severe unilateral lymphedema with a distribution of dark brown, firm nodules and plaques around the knee are features commonly seen with classic variant Kaposi sarcoma.

Differential Diagnosis
Hemangioma, lymphangioma, Kaposi sarcoma, angiosarcoma, melanoma, mycobacterial skin infection.

Pathology
A punch biopsy was performed, and analysis of the tissue sample revealed erythrocyte extravasations with slit-like arrangement, hemosiderin deposition, and hyaline globules. Staining for human herpes virus 8 (HHV-8) was strongly positive, and staining for CD31 (an endothelial cell marker) was also positive. Grocott's methenamine silver staining revealed no fungal elements.

Diagnosis
Classic variant Kaposi sarcoma.

DISCUSSION

This clinical vignette with photographs illustrates a good example of Kaposi sarcoma. Given the patient's country of origin and the absence of a history of organ transplantation or HIV infection, the most likely diagnosis is the classic variant subtype of Kaposi. This classic variant effects men more than women (ratio 15:1), and usually occurs in older men of Mediterranean and Eastern European ancestry.[1] Other subtypes of Kaposi sarcoma include endemic, organ transplantation associated, and epidemic or AIDS related. The endemic or African subtype affects the population of equatorial Africa and is not associated with immunodeficiency. Organ transplantation–related Kaposi can occur with viral transmission from the organ or with reactivation in the recipient. Patients with HIV have a unique susceptibility to Kaposi sarcoma. This AIDS-defining illness occurs 20,000 times more often in patients with HIV than in the general population.[2]

Pathologically, Kaposi sarcoma is a neoplasm of the lymphatic endothelium. Originally identified in 1994, the causative factor HHV-8 is found in >95% of these sarcomas.[3] HHV-8 is a member of the gammaherpesvirus family, which also includes Epstein-Barr virus. These viruses are known for causing lymphoproliferative disorders.[1] The clinical presentation of HHV-8 usually involves infection of vascular and lymphatic endothelial cells and has lytic and latent phases. The latent phase involves production of lymphangiogenic growth factors, leading to a shift in gene profile so that the cell blood vascular endothelial cells resemble lymphatic endothelial cells.[4] Further grow regulation is mediated through latency-associated nuclear antigen, which inhibits p53 activity.[5] The slow disease progression with precedent lymphedema in the patient we describe is very characteristic of the classic variant. The patient was heterosexual, which would place him at lower risk for Kaposi sarcoma than a homosexual man even without identifiable immunosuppression.[6]

After diagnosis the allocation of treatment is difficult due to lack of a fully accepted staging system for classic Kaposi sarcoma. According to a proposed staging system from the *European Journal of Dermatology,* this patient's disease would be classified as stage II infiltrative on the basis of the presence of plaques.[7] Because of the indolent nature of this disease, treatment during stages I and II is best approached with radiotherapy, local excision, elastic stockings, and intralesional chemotherapy with vincristine. Local radiation has been shown to cause a partial response in all patients and a complete response in 88%-98.7%[8] and therefore was felt to be a beneficial option. Our patient was referred to lymphedema and dermatology clinics to discuss local radiation therapy.

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A Familial Form of Wolff-Parkinson-White Syndrome: A Case Report and Management Considerations for Patients with PRKAG2 Mutations

Samim Enayat, MD, Postgraduate Year 2, Internal Medicine
Pamela K. Mason, Assistant Professor of Medicine, Division of Cardiovascular Medicine

Wolff-Parkinson-White syndrome (WPW) is a well-recognized condition caused by an accessory pathway that bypasses the atrioventricular (AV)-nodal system to conduct impulses from the atria to the ventricles. Classic electrocardiogram (ECG) changes include a shortened PR interval and a slurred and broad QRS upstroke or delta wave, caused by rapid ventricular preexcitation down this accessory pathway. Various tachyarrhythmias are associated with WPW, including AV reentrant tachycardia (AVRT), atrial fibrillation, and atrial flutter. Atrial fibrillation and atrial flutter can be life-threatening in patients with WPW, owing to rapid conduction down the accessory pathway. Here we describe a patient with familial WPW who presented with preexcited atrial flutter and subsequently developed complete heart block after receiving procainamide therapy. The clinical course of patients with familial WPW differs from that of patients with other forms of WPW. Over time, familial WPW patients develop conduction disease in both the normal conduction system and the accessory pathway, ultimately leading to complete heart block.[1] These patients can also develop left ventricular hypertrophy (LVH) and congestive heart failure.[2,3] Recognition of this condition is important so that appropriate management can be instituted.

CASE REPORT

A 41-year-old woman with a history of WPW presented to an outside facility after experiencing a sudden onset of palpitations while sitting in her car. Along with the palpitations, the patient reported chest pressure with radiation down her left arm, with associated nausea and vomiting. On presentation, the patient's ECG showed preexcited atrial flutter with a 2:1 conduction pattern with rates of 140-150 beats/min (Figure 1). Given her known history of WPW, the patient was started on a procainamide infusion. The patient developed complete heart block with pauses of 3-4 seconds and symptomatic hypotension (Figure 2). The procainamide infusion was discontinued, and the patient received emergent cardioversion to convert her to sinus rhythm. She declined further

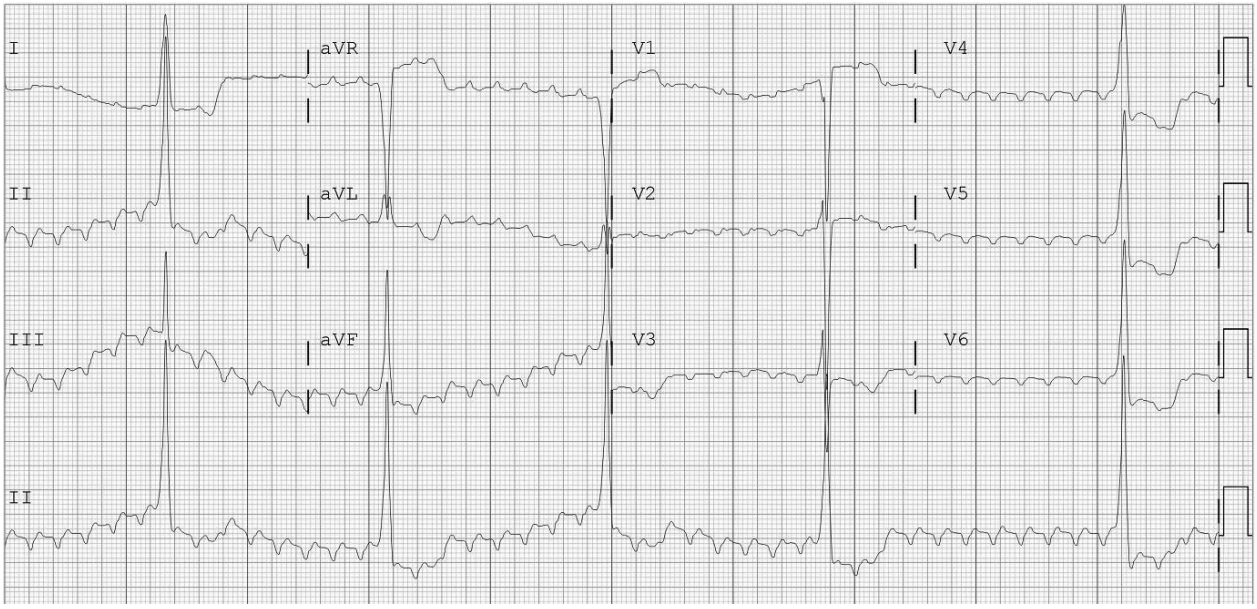

Figure 1. Electrocardiogram obtained at the time of the patient's initial presentation to the outside hospital, demonstrating preexcited atrial flutter with 2:1 conduction to the ventricle.

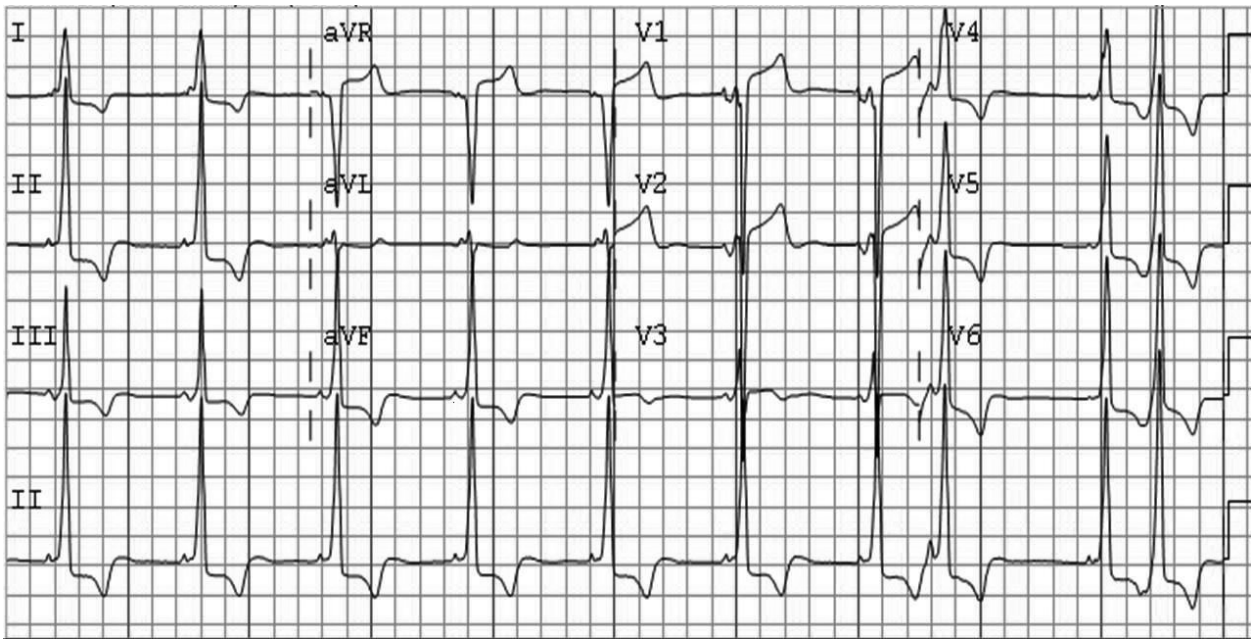

Figure 2. Electrocardiogram after administration of procainamide, demonstrating underlying atrial flutter with complete heart block.

medical care and was discharged. She presented to the Emergency Department of the University of Virginia Health System 3 days later with complaints of shortness of breath, orthopnea, and increasing lower-extremity edema.

The patient's medical history was pertinent for WPW and multiple documented episodes of AVRT, atrial fibrillation, and atrial flutter. She had previously undergone 3 electrophysiology studies, and 1 attempted ablation had been performed 3 years prior to the episode we describe. The attempted ablation was aborted because the patient's accessory pathway was thought to be too close to her native conduction system to be ablated safely. After this procedure she continued to have palpitations. Previous cardiac catheterization had revealed her coronary arteries to be normal.

The patient's family history was significant for a son in whom WPW and AVRT were also diagnosed. He underwent successful electrophysiological study and cryoablation therapy at the age of 15 years. The case patient said she did not know her father very well, but reported that he had coronary artery disease and atrial tachyarrhythmias and had received a pacemaker and died of congestive heart failure at a young age.

Physical examination of the patient revealed heart rate of 40/min, blood pressure of 130/60 mm Hg, and 93% oxygen saturation on room air. The patient weighed 192 pounds, compared to 180 pounds at the outside facility less than a week earlier. The patient had mildly elevated jugular venous distension, and lung examination showed crackles at the bases bilaterally. Cardiac exam revealed a regular rhythm with normal S1 and S2 and no murmurs or rubs. Extremity exam showed 2+ lower extremity edema to the middle tibia bilaterally. The admission ECG demonstrated bradycardia with preexcitation (Figure 3). Chest x-ray revealed interstitial pulmonary edema and small bilateral pleural effusions. Laboratory analysis data were pertinent for an admission troponin of 9 ng/mL, which peaked at 11 ng/mL, and a B-type natriuretic peptide of 597 pg/mL.

The patient was admitted to the Acute Cardiology Service of the University of Virginia Health System for further management. She was continued on her home medications and diuresed with intravenous lasix. Transthoracic echocardiogram showed mild concentric LVH with normal systolic function. Cardiac catheterization revealed normal coronary arteries. The patient's rise in troponins was thought to be secondary to demand ischemia from her tachyarrythmia.

The electrophysiology service was consulted, and a diagnosis of probable familial WPW due to PRKAG2 mutation was made on the basis of the patient's

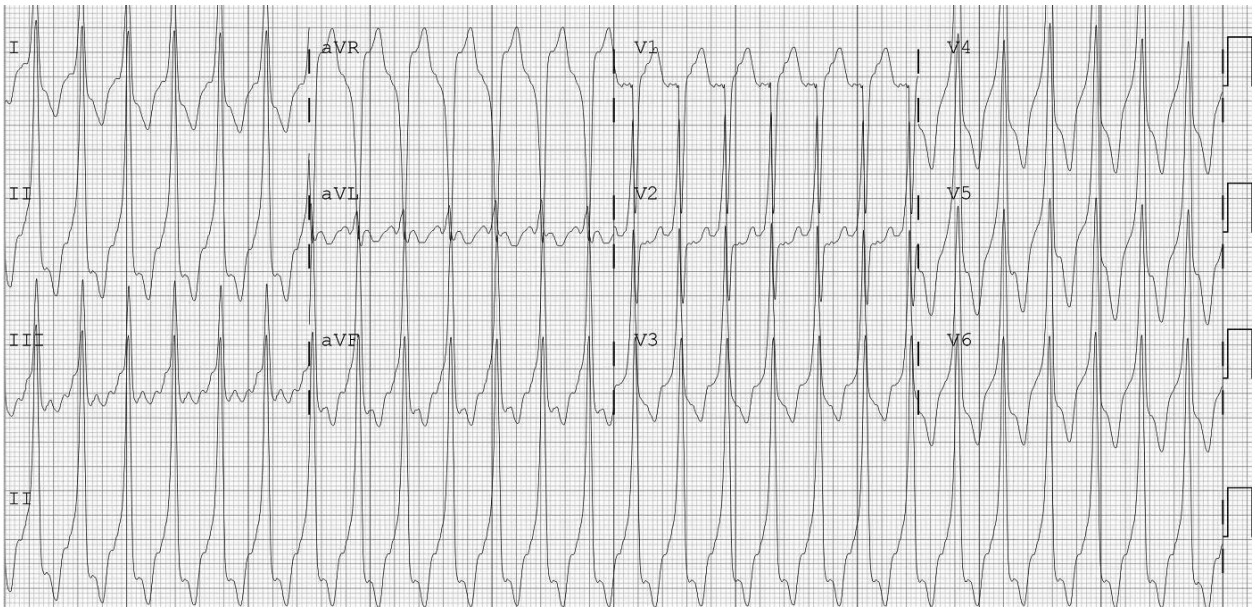

Figure 3. Electrocardiogram (ECG) obtained at the time of admission to the Emergency Department of the University of Virginia Health System. The patient has sinus bradycardia with preexcitation.

family history and clinical course. Procainamide effectively blocked accessory pathways and induced complete heart block in this patient. This outcome demonstrated severe disease of the native conduction system. In addition, the accessory pathway conducted only the atrial flutter in a 2:1 ratio, indicating the accessory pathway was not rapidly conducting. The patient had also developed mild LVH. She received a pacemaker for her conduction system disease and was started on flecainide therapy to control her atrial arrhythmias. At the time of this report the patient had been asymptomatic at follow-up visits. She declined genetic testing for herself and her family, because of economic concerns.

DISCUSSION

In patients with WPW, 3.4% have first-degree relatives with a preexcitation syndrome.[4] In the general population the prevalence of preexcitation syndromes is only 0.15%, so the higher prevalence in first-degree relatives of WPW patients indicates a genetic component to the pathogenesis of WPW. A familial form of WPW has been identified and is inherited in an autosomal dominant fashion.[4] The culprit mutation is in the PRKAG2 gene and is localized on chromosome 7q34-q36, which encodes for the gamma-2 regulatory subunit of AMP-activated protein kinase (AMPK).[1]

AMPK plays a key role in regulating the cellular energy demands in many cell types, including those found in cardiac tissue. It is hypothesized that derangements in the function of AMPK lead to abnormal cellular glycogen accumulation, which results in the variable phenotypes of this cardiac syndrome, including WPW with and without cardiac hypertrophy.[5] In patients with PRKAG2 mutations who develop cardiac hypertrophy, histological examination of the myocardium reveals myocyte enlargement with glycogen-filled vacuoles.[2,3] These histological findings lend credence to the theory that familial WPW is fundamentally a glycogen-storage disease. Nonetheless, the mechanisms whereby PRKAG2 mutations cause accessory pathway formation and conduction system disease are poorly understood and are currently being investigated.

A number of families with PRKAG2 mutations have been identified and reported. Within those families, the phenotype is variable.[6] Patients with familial WPW often present at a young age because of symptomatic palpitations. These patients are prone to develop AVRT and atrial fibrillation, as are patients with nonfamilial WPW.[7] As with the patient we describe here, patients with familial WPW develop disease of their conduction system, usually around the fourth decade of life.[1] Both the native conduction system and the accessory pathway are

involved, and these patients ultimately develop complete heart block and require pacemaker implantation. Phenotypes with and without hypertrophic cardiomyopathy have also been described.[7-9] The risk of sudden cardiac death in this population is relatively undefined, but likely depends on the specific mutation.

Clinical diagnosis of familial WPW requires a high index of suspicion. Particular attention to the family history is important in making the clinical diagnosis. Discovery of a family history of conduction system disease and LVH can be helpful in counseling patients with regard to the probable course of their illness. Genetic testing is available through research institutions and several commercial laboratories and is needed to definitively make the diagnosis. Although definitive diagnosis can be very helpful for the family members of probands, insurance coverage for this testing is not widely available, and the cost is prohibitive for many patients.

There are important management considerations for patients with familial WPW. Young patients with symptomatic palpitations can undergo ablation of the accessory pathway, as did the son of this case patient. As patients age, however, the development of native conduction system disease increases the risk that ablation of the accessory pathway will cause complete heart block. Likewise, treatment with antiarrhythmic medications such as procainamide can induce complete heart block. For this patient, a combination of pacing therapy and antiarrhythmic medication was selected for treatment. Her son was counseled that he will likely develop conduction system disease and LVH as he ages. There are few data available to guide medical treatment for LVH associated with this condition. In general, standard medical therapies are employed, such as angiotensin-converting enzyme inhibitors, beta blockers, and diuretics. If this patient's LVH worsens, she may no longer be a candidate for flecainide therapy, and an alternate antiarrhythmic medication may be selected.

CONCLUSION

WPW is a clinical syndrome that most physicians will encounter in their training or practice. The importance of recognizing and understanding the familial form of WPW syndrome is made apparent in the clinical vignette presented here. Typical antiarrhythmic medications that are effective and indicated in the treatment of WPW syndrome are potentially harmful in patients with the PRKAG2 mutation. In addition, these patients are at risk for ventricular hypertrophy and heart failure, and require monitoring and treatment for these problems. Clearly, further studies are required to elucidate the complex genetics and pathogenesis of familial WPW syndrome.

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Baking Soda for Dyspepsia: Therapeutic or Toxic?

An Hong (Barry) Bui, BS, Medical Student
Srikant Nannapaneni, MD, MPH, Assistant Professor of Medicine, Division of General Medicine, Geriatrics, and Palliative Care

For many years, baking soda (sodium bicarbonate) has been used as a home remedy for conditions such as dyspepsia, diaper rash, stomatitis, and viral syndromes.[1] The use of baking soda remains prevalent despite the availability of dyspepsia treatment agents such as H2 blockers and proton-pump inhibitors. The high cost of these medications in the setting of limited access to health care and low socioeconomic status contributes to the continued use of home remedies such as baking soda. We present a case of chronic ingestion of baking soda as a home remedy, and focus on the presentation and management of the patient's resultant acid-base and electrolyte abnormalities.

CASE REPORT

A 53-year-old African-American man with a history of gastroesophageal reflux disease and severe erosive esophagitis presented to the emergency department with a 3-day history of hiccups and a 6-month history of nausea and vomiting. The patient complained of odynophagia, dysphagia to solids, and postprandial vomiting. He denied hemoptysis, hematemesis, diarrhea, constipation, shortness of breath, fever, chills, and night sweats. The patient's home medications included lansoprazole and ferrous sulfate. The patient reported drinking alcohol twice a month but denied tobacco or illicit drug use. He said he was unemployed and lived with his mother.

Physical examination revealed that the patient's vital signs were remarkable for orthostatic hypotension (131/76 mm Hg supine, 83/47mm Hg standing) and tachycardia (pulse 90 beats/min supine and 99 beats/min standing). Head and neck exam revealed asymmetric pupils (right 4 mm, left 2.5 mm). Chest and cardiac exams were unremarkable. An abdominal exam revealed hypoactive bowel sounds. The patient did not have peripheral edema, and peripheral pulses were normal. His neurologic exam was unremarkable except for an afferent pupillary defect. Results of serum chemistry tests were significant for sodium 118 mEq/L, potassium 2.5 mEq/L, chloride 52 mEq/L, bicarbonate 44 mEq/L, creatinine 4.4 mg/dL, urea 88 mg/dL, glucose 105 mg/dL, ionized calcium 6.9 mg/dL, magnesium 2.2 mg/dL, and phosphorus 6.8 mg/dL. The patient's white blood cell count was 16,800 cells/mm^3, and hemoglobin was 14.7 mg/dL. Arterial blood gas analysis showed a pH of 7.56, a P_{CO2} of 55.2 mm Hg, and a P_{O2} of 67.3 mm Hg. Concern for an intracranial process as a cause of the patient's hiccups and afferent papillary defect prompted us to perform a computerized tomography of the head without contrast. The results of this examination were unremarkable. Electrocardiogram showed normal sinus rhythm with a prolonged QTc of 563 milliseconds. Chest radiograph revealed no evidence of cardiac or pulmonary disease.

After reviewing the patient's laboratory data, we questioned him about possible baking soda use. He admitted to ingesting a mixture of baking soda, vinegar, and water as a remedy for dyspepsia and hiccups. The patient reported that this mixture was a traditional home remedy passed down by his father and used for treatment of epigastric pain and hiccups.

DISCUSSION

Sodium bicarbonate is indicated for treatment of conditions including metabolic acidosis, gastric hyperacidity, urinary alkalinization, overdose of tricyclic antidepressants and salicylates, and unlabeled use for prevention of contrast-induced nephropathy.[2] Ingestion of sodium bicarbonate in the setting of volume depletion can lead to severe metabolic alkalosis.

Metabolic alkalosis is commonly caused by diuretic therapy or by gastric secretion loss due to vomiting or nasogastric suction. Normally, the kidney preserves acid-base balance by means of bicarbonate reabsorption in the proximal tubule and generation of bicarbonate in the distal nephron.[3] In healthy individuals, the addition of alkali will not typically lead to metabolic alkalosis, because the

kidneys increase the rate of bicarbonate excretion.[3] Ingestion of 140 g of sodium bicarbonate daily for up to 3 weeks has been shown to have no deleterious effect on the kidneys in healthy individuals.[4] To maintain metabolic alkalosis, there must be a coexisting impairment of renal bicarbonate excretion.

Factors influencing bicarbonate excretion include reduction in glomerular filtration rate and, more importantly, increased tubular reabsorption of bicarbonate. Conditions such as reduced arterial blood volume, chloride depletion, hypokalemia, and hyperaldosteronism lead to increased bicarbonate reabsorption in the renal tubules and are essential in maintenance of metabolic alkalosis.[5] Our patient's 6-month history of vomiting with resultant volume contraction and loss of hydrogen ions resulted in a lower effective arterial blood volume and glomerular filtration rate. These adverse consequences led to prerenal acute kidney injury, which prevented effective diuresis, further worsening his metabolic alkalosis.

The pathophysiology of metabolic alkalosis can be divided into generation and maintenance phases. Generation of metabolic alkalosis in our patient can be attributed to sodium bicarbonate ingestion combined with loss of protons from vomiting. On the other hand, the maintenance of metabolic alkalosis was due to reduced arterial blood volume and coexisting chloride depletion from prolonged vomiting.

The pathophysiology of increased bicarbonate reabsorption in chloride depletion is not clearly understood. One postulated mechanism is that the decrease in the delivery of chloride ions to the cells of the macula densa in the renal tubules leads to an increase in renin secretion, which activates the renin-angiotensin-aldosterone system.[5] The resultant rise in aldosterone secretion leads to increased proton secretion into the lumen of the collecting tubules and results in metabolic alkalosis. Furthermore, depletion of chloride ions results in a loss of electronegativity between the intracellular and extracellular fluid compartments, a situation that favors the reabsorption of bicarbonate in the collecting tubules to maintain electroneutrality.[6] The resulting alkalemia stimulates the H-K-ATPase exchange pumps in the collecting tubules to shift potassium intracellularly in exchange for hydrogen, worsening the hypokalemia.[7]

Prolonged alkalemia can cause hypoxemia, decreased cerebral and myocardial blood flow, obtundation, dysrhythmias, neuromuscular excitability, and a left shift in the oxygen dissociation curve.[6] Severe metabolic alkalosis is associated with high risk of mortality. Reported data suggest that in patients with a pH of 7.55 the mortality rate is 45% and in those with a pH of 7.65 or greater the mortality rate is as high as 80%.[8] A lethal complication of bicarbonate ingestion is gastric rupture due to the release of dissolved carbon dioxide gas formed by the reaction of bicarbonate and gastric acid.[9] Luckily, our patient was mixing baking soda with vinegar and water, causing the release of carbon dioxide prior to ingestion.

Our patient also presented with hyponatremia, which was likely due to chronic emesis and volume depletion leading to activation of antidiuretic hormone. In addition, nausea is another strong stimulant for antidiuretic hormone secretion, further worsening hyponatremia.

Workup of metabolic alkalosis in this patient included measurement of serum chemistries and urinary electrolytes including sodium, chloride, and potassium. Low urinary chloride (<10 mEq/L) characterizes alkalosis in which chloride depletion predominates and indicates chloride-responsive alkalosis. Checking arterial blood gas is also helpful for assessing the severity of alkalosis and diagnosing mixed acid-base disorders. Severe metabolic alkalosis can induce hypoventilation to produce a compensatory respiratory acidosis, as was evident in our patient on the basis of his arterial blood gas values.

Treatment of metabolic alkalosis is primarily achieved by intravenous hydration and correction of electrolyte abnormalities. Our patient received volume resuscitation with normal saline. The rate of normal saline infusion was calculated for the goal of correcting the patient's serum sodium level by 8-12 mEq/24 hours. The resulting increase in intravascular volume decreases the stimulus for renal bicarbonate reabsorption. Furthermore, the increase in distal chloride delivery increases bicarbonate secretion via chloride-bicarbonate exchangers in type B intercalated cells in cortical collecting tubules.[7] The patient was also treated with oral potassium replacement. Increase in serum potassium stimulates exchange of potassium for intracellular hydrogen to maintain electroneutrality and to buffer excess extracellular bicarbonate.

With initiation of therapy, the patient's metabolic alkalosis and hypokalemia resolved by day 3, and hyponatremia resolved by day 7. The patient's creatinine progressively improved throughout his hospital stay, dropping from 4.4 mg/dL at admission to 2.1 mg/dL by the day of discharge.

Evaluation for dysphagia with a barium swallow revealed narrowing of the cervical esophagus. Endoscopic examination confirmed cervical esophageal narrowing, severe esophagitis, large hiatal hernia, and an esophageal plaque. Biopsy revealed squamous papilloma without dysplasia. Cervical esophageal dilatation under fluoroscopy was performed for relief of dysphagia. The patient was discharged to home on day 7 with instructions to stay hydrated and to never use baking soda. At a follow-up visit 7 days after discharge, the patient was found to have normal electrolytes and creatinine levels.

SUMMARY

Sodium bicarbonate ingestion in the setting of severe volume depletion can lead to metabolic alkalosis. Our patient failed to report baking soda use and was unaware of the danger of this home remedy for dyspepsia and hiccups. Obtaining a thorough history regarding the use of home remedies is recommended owing to the prevalence of baking soda use for a variety of illnesses, especially in populations with limited health care access and low socioeconomic status. Management of metabolic alkalosis due to baking soda ingestion includes hospitalization, aggressive hydration with intravenous fluids, and replacement of electrolytes. Furthermore, patients need to be educated about the danger of baking soda ingestion.

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Catastrophic Antiphospholipid Antibody Syndrome Presenting with Abdominal Pain, Chest Pain, and Multiple Skin Lesions in a 20-Year-Old Woman: Case Report and Review of the Literature

Anshul Aggarwal, MD, Postgraduate Year 2, Internal Medicine
Matt Goodman, MD, Associate Professor of Internal Medicine, Division of General Medicine, Geriatrics, and Palliative Care

Catastrophic antiphospholipid antibody syndrome (CAPS) was first described in 1992 as a rapidly progressive fatal variant of antiphospholipid antibody syndrome. This syndrome presents with multiorgan failure and occurs predominantly in females. We report a case of CAPS in a female patient who presented with myocardial infarction, hepatic venous occlusion, and multiple cutaneous embolic events. We also present a review of pertinent literature.

CASE REPORT

The patient was a 20-year-old woman who presented to an outside hospital with a 3-month history of sharp, intermittent abdominal pain that did not radiate to other locations. The pain was diffuse, with no relation to liquid or solid intake. The patient reported that within the 4 weeks prior to presentation she had suffered worsening pain in her right upper quadrant with some associated nausea and vomiting. The discomfort had progressively worsened until the patient could no longer tolerate consumption of anything by mouth, and she had unintentionally lost 30 to 40 pounds during the past 3 months. She did not complain of fevers, chills, or any other associated symptoms.

At the outside hospital, results of the patient's laboratory tests revealed significant findings: albumin 2.3 g/dL, total bilirubin 1.3 mg/dL, alkaline phosphatase 84 units/L, aspartate aminotransferase 97 units/L, and alanine aminotransferase 79 units/L. Results of a hepatobiliary iminodiacetic acid scan were normal, and right upper quadrant ultrasound with Doppler showed patent vasculature as well as a normal gallbladder. A computed tomographic scan of the patient's abdomen showed multiple heterogenous left liver lobe lesions, and the patient was then transferred to the University of Virginia Health System.

The patient's medical history included systemic lupus erythematosus complicated by antiphospholipid antibody syndrome, and bilateral lower extremity deep venous thromboses. Her home medications included hydroxychloroquine 200 mg daily, mycophenolate mofetil 1000 mg in the morning and 500 mg in the evening, prednisone 30 mg daily, and warfarin sodium (Coumadin) 3 mg at night.

The patient's physical examination revealed normal vital signs. Her skin exam showed a 2 x 2–cm cutaneous thrombosis over her left malar region (Figure 1). She had moderate-to-severe abdominal pain on palpation in all 4 quadrants. Her heart examination revealed regular rate and rhythm with normal S1 and S2.

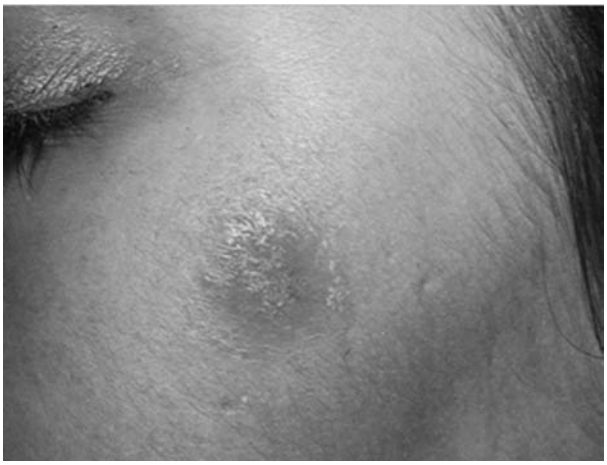

Figure 1. Left-sided 2 ´ 2–cm malar rash visible on the patient's face at the time of admission to the University of Virginia Health System.

Laboratory tests performed when the patient was admitted to our institution showed aspartate aminotransferase of 184 units/L, an alanine aminotransferase of 158 units/L, and a total bilirubin of 0.8 mg/dL. Amylase and lipase values were normal. The patient's antinuclear antibody was

positive, with a titer of 1: 640, and her international normalized ratio was 2.5.

Results of liver function tests continued to rise, with a peak aspartate aminotransferase of 356 units/L and peak aspartate aminotransferase of 331 units/L. Magnetic resonance imaging with angiography of the abdomen was performed and showed multiple hyperenhancing nodular foci consistent with an occluded left hepatic vein, findings consistent with a diagnosis of Budd-Chiari syndrome.

During the course of her hospitalization, the patient developed superficial thromboses over her left ear, left cheek, and left fifth toe, which became necrotic.

On hospital day 6, the patient developed acute substernal chest pain without associated shortness of breath or diaphoresis. Her electrocardiogram showed ST elevations in the inferior leads II, III, and aVF as well as in the lateral leads V4 to V6, with a troponin peak of 5.04 ng/mL. A transthoracic echocardiogram showed no obvious wall-motion abnormality, and she had a normal left ventricular ejection fraction of 65%.

Table 1. Diagnostic Criteria of Antiphospholipid Antibody Syndrome*

1. Evidence of involvement of 3 or more organs, systems, and/or tissues.
2. Development of manifestations simultaneously or within 1 week.
3. Confirmation by histopathology of small vessel occlusion in at least 1 organ or tissue.
4. Laboratory confirmation of the presence of antiphospholipid antibodies.

*Patients are considered to have definite catastrophic antiphospholipid antibody syndrome if they fulfill all 4 diagnostic criteria. Patients are considered to have probable antiphospholipid antibody syndrome if they meet all 4 criteria but have involvement of only 2 organs or systems, if they meet all 4 criteria but have only a single positive determination for antiphospholipid antibodies, or if they meet the first and last criteria, but only 1 of the 2 middle criteria. Adapted from Asherson et al.[1]

We diagnosed the patient's illness as CAPS with multiorgan failure, a diagnosis that was confirmed by positive results of tests for antiphospholipid antibodies. Diagnostic criteria are given in Table 1. The patient was started on anticoagulation and corticosteroids. Her superficial thromboses continued to worsen, however, and she complained of increasing abdominal pain with rising transaminases. She was subsequently started on intravenous immunoglobulin (IVIG) for a 5-day course.

When the patient developed an ST-elevation myocardial infarction, cardiac catheterization was deferred owing to potential risk of further propagation of thromboses with placement of an arterial sheath. Instead, she was medically managed with aspirin and metoprolol, and continued on anticoagulation.

After the patient completed IVIG for 2 days, her transaminases normalized and her abdominal pain subsided. She no longer complained of chest pain, and her cutaneous thromboses began to resolve. Our patient was discharged after having completed 5 days of IVIG. She then underwent close follow-up in the rheumatology clinic. Her prednisone was increased to 60 mg daily.

DISCUSSION

CAPS is an increasingly recognized entity. The cascade of microvascular and macrovascular thromboses that leads to CAPS usually begins with an identifiable trigger in approximately 60% of patients.[1] The most prevalent triggers of CAPS include viral upper respiratory tract infections and urinary tract infections, immunizations with influenza and yellow fever, major and minor surgical procedures, traumas, and malignancies, particularly lymphoma.[1]

The pathogenesis of CAPS remains unclear. It has been hypothesized that this syndrome results from increasing fibrinogen production along with consumption of anticoagulants protein C, protein S, and antithrombin III.

CAPS can present with a wide variety of signs and symptoms. Intrarenal manifestations are the most common manifestations of CAPS, with 71% of patients affected with hypertension, renal thrombotic microangiopathy, and renal infarcts. The second most commonly affected organ system is the lung, with lung involvement occurring in approximately 64% of patients.[2] Patients with CAPS can develop pulmonary embolism, microinfarcts, and resultant pulmonary hypertension, as well as pulmonary edema and acute respiratory distress

syndrome. Approximately 51% of patients have cardiac involvement, presenting with mitral or aortic valvular stenosis, intracardiac thrombus, or rarely, myocardial infarction. Intrahepatic involvement occurs in 33% of patients and is usually manifested by thrombotic events.[2]

Thrombocytopenia is present in nearly 60% of patients with CAPS. One third of these patients have evidence of hemolysis, with 20% having features of disseminated intravascular coagulation.[3] IgG anticardiolipin antibodies are usually strongly positive, whereas IgM elevations are less common.[3] Mortality in patients who develop CAPS is approximately 50%, and CAPS patients frequently require intensive care unit monitoring.[3] First-line therapy to prevent further thromboses includes systemic anticoagulation with intravenous heparin and corticosteroids to prevent cytokine release.[1] IVIG or plasma exchange may be effective in refractory cases. IVIG for a total course of 5 days can specifically help in decreasing total antibody levels. Plasma exchange allows for removal of several cytokines and can help decrease inflammation. Some clinicians will administer IVIG in combination with plasma exchange for increased effectiveness.

CONCLUSION

CAPS is a serious complication of antiphospholipid antibody syndrome that usually presents with an identifiable trigger. Renal and pulmonary manifestations are the most common signs and symptoms; however, virtually all organ systems can be affected. The first-line therapies remain anticoagulation with heparin and administration of corticosteroids. Second-line therapies include plasma exchange or a 5-day course of IVIG.

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Toxic Epidermal Necrolysis: A Case Report and Review of Current Management

Matthew J. Chung, BS, Medical Student
Alexandra Kadl, MD, Postgraduate Year 3, Internal Medicine
Heidi Zapata, MD, PhD, Postgraduate Year 2, Internal Medicine
George Verghese, MD, Associate Professor of Medicine, Division of Pulmonary and Critical Care Medicine

Toxic epidermal necrolysis (TEN) is an acute severe drug reaction characterized by widespread keratinocyte apoptosis leading to mucous membrane erosions and epidermal necrosis. The annual incidence of TEN is estimated to be 1 to 2 cases per million per year, and the reported mortality rate varies from 30% to 50%.[1,2] Although the pathogenesis of TEN has become more clear since the syndrome was first described by Lyell in 1956, no single treatment has emerged as being efficacious and superior to supportive care.[3] The current literature lacks reports of randomized controlled trials for specific treatments and consists only of numerous case reports and noncontrolled clinical studies. We describe a case of TEN in a 61-year-old woman and review the current treatment options.

CASE REPORT

The case patient was a 61-year-old white woman with stage IIIB non–small cell lung cancer treated with chemotherapy, radiation therapy, and a later round of palliative chemotherapy for local recurrence. The patient presented to an outside hospital with an erythematous maculopapular rash of 2 days duration. The rash was diffuse but spared the patient's palms and the soles of her feet. She also reported a sore mouth and throat and said she was unable to eat and drink because of the pain. Pertinent negative findings included absence of fever, chills, cough, and conjunctivitis. The patient had begun taking ciprofloxacin 500 mg twice a day for a urinary tract infection and developed the rash the morning after taking 2 doses. In addition, she had received a third cycle of gemcitabine, pemetrexed, and carboplatin 4 days prior to the development of the rash. The patient had received ciprofloxacin in the past without incident. She had no known prior drug allergies. On admission, her medications included ciprofloxacin, mirtazapine, transdermal fentanyl, acetaminophen, hydrocodone, lorazepam, inhaled ipratropium bromide and albuterol sulfate, amitriptyline, lisinopril, hydrochlorothiazide, and folic acid.

The patient's medical history included hypertension, hyperlipidemia, depression, anxiety, and chronic pain secondary to her malignancy. She had a 30 pack-year history of smoking but had quit 12 years prior to this admission. Results of her family history, social history, and review of systems were otherwise unremarkable.

On admission to the outside hospital, the patient was afebrile. Her blood pressure was 108/60 mm Hg and her pulse rate was 88 beats/min. Physical examination revealed that she appeared tired but was not in acute distress. She had severe oral mucositis with sloughing of her lips and oral mucosa. No conjunctivitis was noted. Cardiopulmonary and abdominal examination findings were normal. An erythematous maculopapular rash with blisters and pustules was present on the patient's face, axillae, trunk, groin, and extremities. The rash did not involve the palms of her hands or the soles of her feet. She was appropriately responsive and her neurological exam showed no focal deficits.

Laboratory evaluation revealed the following: sodium 142 mmol/L, potassium 4.2 mmol/L, chloride 103 mmol/L, bicarbonate 13 mmol/L, blood urea nitrogen 147 mg/dL, creatinine 10.6 mg/dL, and glucose 104 mg/dL. Calcium was 4.8 mg/dL and phosphorus 13.3 mg/dL. Total protein was 6.5 mg/dL, albumin 2.8 g/dL, total bilirubin 0.9 mg/dL, lactate dehydrogenase 1114 U/L, alkaline phosphatase 190 U/L, alanine aminotransferase 32 U/L, and aspartate aminotransferase 68 U/L. White blood cell count was 0.9 k/μL, hemoglobin 11.1 g/dL, hematocrit 31.3% (mean corpuscular volume 87.9 fL), and platelet count 98 k/μL. Prothrombin time was 15.7 seconds, partial thromboplastin time 35 seconds, and international normalized ratio

1.27. A culture of a pustule on the patient's chest showed no growth.

In the emergency department, the patient received 1 L of normal saline and dexamethasone 20 mg intravenously before being transferred to the inpatient service. Ciprofloxacin, mirtazapine, lisinopril, and hydrochlorothiazide were held. Despite ongoing fluid resuscitation, during the next 2 days the patient had persistent acute kidney injury that was attributed to acute tubular necrosis. She was started on intermittent hemodialysis. The patient remained pancytopenic and transfusion dependent secondary to her recent chemotherapy, and she was started on a daily dose of filgrastim 300 µg subcutaneously. During the first 48 hours, the patient was treated with methylprednisolone 60 mg daily for presumed TEN. A subsequent skin biopsy showed subepidermal bulla formation with overlying confluent necrosis of the epidermis and an underlying dermal infiltrate of lymphocytes, eosinophils, and neutrophils, findings consistent with TEN (Figure 1). Methylprednisolone was discontinued at this point (hospital day 3).

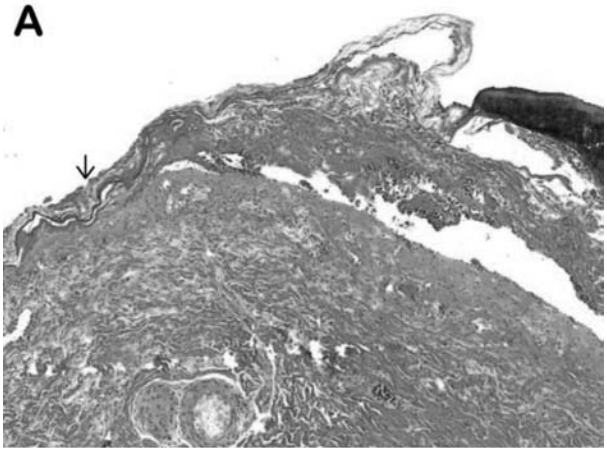

Figure 1. A skin biopsy sample showing subepidermal bulla formation with overlying confluent necrosis of the epidermis and an underlying dermal infiltrate of lymphocytes, eosinophils and neutrophils. Remaining viable epidermis is present at the left margin of the picture in (A) (â). Hematoxylin-eosin stain, (A) original magnification ×40, (B) original magnification ×100. Pictures courtesy of Dr. Jochen Schafer, MD.

The patient was transferred to the oncology service at the University of Virginia Health System on hospital day 5 for further management. On presentation to our institution, the patient had trouble speaking and eating secondary to pain, but had no other complaints. Pertinent findings on exam included absence of fever, multiple oral ulcers with hemorrhagic crusts on her lips, coarse rhonchi throughout her lung fields bilaterally, and a diffuse erythematous maculopapular rash involving approximately 80% of her total body surface area with sloughing in some areas (Figure 2). She had a positive Nikolsky sign (flaccid bulla that sloughed off with slight sheer pressure). The patient was transferred the next day to the medical intensive care unit because of increased respiratory secretions and concern for inability to protect her airway.

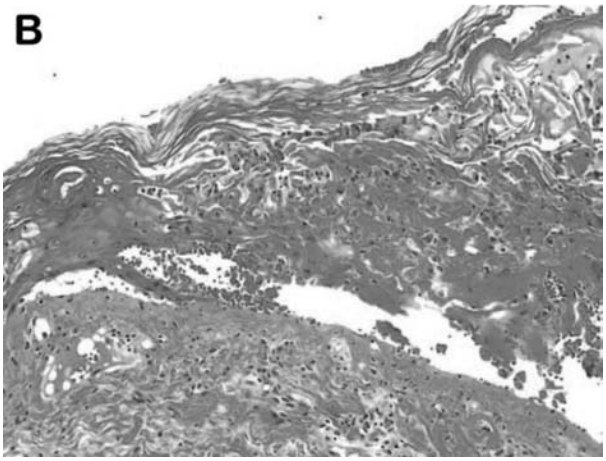

Figure 2. Mucosal erosions and hemorrhagic crusts are apparent in the patient's mouth (A, B). A diffuse erythematous maculopapular rash with some confluent areas is apparent on the patient's legs (C, D). The rash progressed to flaccid bulla that sloughed off with slight sheer pressure (positive Nikolsky sign). A and C, day 10 after the patient received 2 doses of ciprofloxacin; B and D, day 12 after ciprofloxacin.

In the medical intensive care unit, the patient received supportive care with fluid and electrolyte replacement, meticulous wound care, nutritional support, intermittent hemodialysis, and fentanyl for pain management. The patient required intubation 2 days after transfer to the medical intensive care unit because of copious secretions, hypoxia, and inability to protect her airway. The patient also developed hypothermia and hypotension requiring vasopressor therapy. Because of concern for sepsis, the patient was started on broad-spectrum antimicrobial coverage with vancomycin, cefepime, and metronidazole. Bronchoscopy showed patchy mucosal ulcerations in the tracheobronchial tree and cultures of bronchial washings grew abundant fungal organisms consistent with *Candida* species. Fluconazole was added to the patient's antimicrobial coverage. The patient received intravenous immune globulin (IVIG) 1g/kg for

treatment of toxic epidermal necrolysis on hospital day 8. Unfortunately, the IVIG had to be stopped after 1 dose because of possible hemolysis. The patient's clinical status continued to decline. She was transitioned to comfort care and died on hospital day 12.

DISCUSSION

The gold standard for the diagnosis of TEN is a biopsy sample of affected skin showing the histopathological findings of necrotic keratinocytes in the epidermis progressing to confluent full-thickness epidermal necrosis and formation of subepidermal bullae.[4] Epidermal necrosis is the pathognomonic finding in TEN, distinguishing it from clinically similar acute exfoliative and pustular skin eruptions such as staphylococcal scalded skin syndrome, linear IgA dermatosis, paraneoplastic pemphigus, acute graft-versus-host disease, pemphigus, and acute generalized exanthematous pustulosis.[5]

Prognosis in TEN can be determined by assessing 7 independent risk factors included in a severity-of-illness score referred to as the severity of illness score for toxic epidermal necrolysis syndrome (SCORTEN) (Table 1).[6] One point is given for each independent risk factor that is present. The patient we describe had 6 of these 7 independent risk factors, portending a predicted mortality of 90% (Table 2).[6]

Table 1. Independent Risk Factors in TEN

Risk Factor
Age ≥40 years
Associated malignancy
Heart rate ≥120 beats/min
Body surface area involved at day 1 >10%
Blood urea nitrogen >28 mg/dL
Serum glucose >252 mg/dL
Serum bicarbonate <20 mmol/L

Table 2. Predicted Mortality based on SCORTEN Score

SCORTEN	Mortality
0-1	3.2%
2	12.1%
3	35.3%
4	58.3%
≥5	90.0%

The first treatment in the management of TEN is prompt withdrawal of the offending drug. In general, the faster the offending drug is removed, the better the prognosis. Unfortunately, this has been found not to be true if the drug has a long half-life.[7] In cases of polypharmacy, identifying the causative drug can be difficult, and all potential causative drugs should be discontinued. The most common culprits associated with TEN are antibiotics (especially sulfonamides), nonsteroidal antiinflammatory drugs, anticonvulsants, and allopurinol.[8] The offending drug is usually one that has been newly administered within 4 weeks of presentation.[9] In the patient described here, the most likely causative agent was ciprofloxacin, because this drug was started 1 day prior to development of the rash. Several case reports have described an association between ciprofloxacin and TEN, with both fatal and nonfatal cases reported.[10-13]

Early admission to a burn unit has been shown to reduce the risk of infection and reduce mortality in TEN patients.[4] As with burn patients, loss of the epidermis in TEN necessitates meticulous adjustment of fluid and electrolyte balance, nutritional support with a high-protein diet, strict thermoregulation, adequate analgesia, and early detection and treatment of infection. Debridement of necrotic epidermis is necessary because it may serve as a nidus for infection. Coverage of the exposed dermis with artificial membranes, such as Biobrane®, has been shown to reduce pain, decrease the risk of sepsis and facilitate reepithelialization, with no reported adverse side effects.[2] There is no role for prophylactic antibiotics, and these agents should be initiated only when signs of sepsis occur.[14] Vigilant care of the eyes is necessary, including the use of lubricants and steroid drops and lysis of adhesions with a sterile glass rod.[15]

In addition to the above general supportive measures, which are widely accepted, several adjuvant treatments have been employed that remain controversial. These treatments are aimed at the underlying immunopathology of TEN, which includes drug-induced clonal expansion of $CD8^+$ cytotoxic T lymphocytes that express perforin and granzyme B, increased levels of TNF-α, and upregulation of death-receptor pathways involving Fas and Fas ligand.[1,16] Collectively, these immunological events lead to widespread activation of apoptosis in keratinocytes and subsequent necrosis and sloughing of the epidermis.

In an attempt to suppress these immunological pathways, several agents have been used, including corticosteroids, cyclophosphamide, cyclosporine, TNF-α inhibitors, and IVIG. To date, the only randomized controlled trial conducted involved thalidomide, a potent inhibitor of TNF-α, and the study was stopped because of significantly higher mortality in the thalidomide group compared with the placebo group.[17] Use of corticosteroids is controversial, with some uncontrolled studies showing benefit and others showing harm.[18-20] The overall stance of the literature is that corticosteroids should be avoided because of the increased risk of sepsis; however, corticosteroids may have some role in treatment if used during the very early erythroderma stage for no longer than 48 hours.[21] A few small studies have shown benefit with the use of cyclophosphamide, but the paucity of data makes this drug currently experimental at best.[22,23] Cyclosporine has been used with some benefit at a dose of 3 mg/kg per day administered every 12 hours.[24] Again, larger-scale studies are needed before cyclosporine can be recommended as a treatment for TEN.

A larger body of data have been reported on the use of IVIG. A review article for which a literature search from 1995 to 2006 was performed identified 3 prospective studies, 9 retrospective studies, and 2 case series that collectively showed that IVIG administered at an average dose of 0.8 ± 0.4 g/kg per day for a mean duration of 4.0 ± 1.0 days stopped progression of TEN and led to rapid reepithelialization in most cases.[25] Although these results seem promising, this study has limitations in providing definitive support for the use of IVIG. Large, multicenter, long-term studies are needed to provide more conclusive data. IVIG, which consists mainly of IgG from pooled normal human plasma, was initially developed for the treatment of various immunodeficiencies. The proposed utility of IVIG in TEN derives from the presence of anti-Fas immunoglobulins in IVIG that interfere with Fas-Fas ligand interactions and thereby attenuate keratinocyte apoptosis. Unfortunately, there is wide batch-to-batch variation in the anti-Fas activity of different IVIG preparations, which may account for its variable success in reported cases.[2] Serious adverse effects of IVIG include aseptic meningitis, acute renal failure, stroke, myocardial infarction, other thrombotic complications, anaphylaxis, and hemolytic anemia.[26,27] The patient we describe developed severe anemia after receiving 1 dose of IVIG.

In summary, the patient described was managed with general supportive care and immunosuppression with short-term, high-dose intravenous corticosteroids and IVIG. Given the severity of the patient's illness and poor prognosis as determined by the SCORTEN score, these measures were unable to prevent mortality.

CONCLUSION

Despite advancements in knowledge of the underlying immunopathological mechanisms of TEN, the low incidence of this rare disease and the large numbers of cases needed to reach statistical significance in a clinical study make assessment of specific treatments difficult. The lack of an animal model further confounds this problem. The case reports and noncontrolled clinical studies available in the literature do not provide sufficient evidence to confidently support any adjuvant treatment other than general supportive care. At present, the most promising additional treatment appears to be immunosuppression with short-term, high-dose intravenous corticosteroids or IVIG. Other immunomodulators such as cyclophosphamide and cyclosporine have not been studied adequately to warrant recommending their use. Although not yet developed, a specific anti-Fas ligand antibody may prove more successful in the treatment of TEN.

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An Unusual Case of Trigeminal Neuralgia

Benjamin Heyman, BS, Medical Student
Jason Gardenier, BA, Medical Student
Mark E. Williams, MD, Professor of Medicine, Division of General Medicine, Geriatrics, and Palliative Care

Trigeminal neuralgia is a condition that has long been observed, and reports indicating medical understanding of this condition date back nearly 2 thousand years. The first description of trigeminal neuralgia, by Areteus of Cappadoccia, appeared in the second century AD.[1] In London in 1766 a more detailed description was made by the English physician John Fothergill, and the disease was known as Fothergill's disease for nearly a century afterward.[3,4] In 1934, the US neurosurgeon Walker Dandy reported trigeminal neuralgia to be most often caused by vascular compression. The disease has a prevalence of 4.5 in 100,000, and the prevalence increases with age. Women are affected twice as frequently as men.[5] Here we report an unusual case of trigeminal neuralgia with neurovascular compression, in which the ophthalmic (V1) branch of the trigeminal nerve was mainly affected, as opposed to the more common finding, in which the maxillary (V2) or mandibular (V3) branch is involved.

CASE REPORT

The case patient was a very pleasant 90-year-old woman who presented to a clinic in a lifecare community to be evaluated for occasional right-sided facial pain precipitated by opening her mouth widely. The patient stated that the pain usually occurred with eating and chewing solid foods. She described the pain as short and sharp, lasting a few seconds and then stopping, as if it were a bolt of lightning. It occurred first over her medial eyebrow and then radiated around her right eye. She denied pain in all other areas of her face. The patient also denied fever, chills, night sweats, chest pain, shortness of breath, and a history of herpes zoster reactivation.

The patient's medical history was significant for a left mastectomy for breast cancer in 2003. She has a history of endometrial polyps, pernicious anemia, and cataract extraction. Medications included a long history of bisphosphonate use. A full review of systems was otherwise noncontributory with regard to her presenting illness.

On physical examination the patient was in no apparent distress. Her vital signs included blood pressure 132/90 mm Hg, a regular pulse of 74 beats/min, respirations 18/min and unlabored, oxygen saturation 99% on room air, temperature 36°C, and weight 42.5 kg. Examination of the patient's skin did not reveal any lesions. A focused head and neck exam revealed a very subtle right-sided infraorbital facial droop. The patient had no trigger that precipitated pain, and she denied pain occurring in response to palpation. The remainder of the head and neck exam revealed no remarkable findings.

The patient was scheduled for magnetic resonance imaging (MRI) of the head with and without contrast to follow the course of the trigeminal nerve and look for a possible tumor, as well as to check for signs of osteonecrosis. In the interim, the patient was placed on carbamazepine for symptomatic relief. Baseline laboratory studies included a complete blood count and liver function tests to monitor for carbamazepine toxicity. Results of these tests showed no abnormalities. The findings of the MRI showed that the course of the right superior cerebellar artery was tortuous, passing between the dorsal root entry zone of the right fifth trigeminal nerve and the pons. The right superior cerebellar artery abutted and compressed the right fifth trigeminal nerve. There was no evidence of tumors, hemorrhage, hydrocephalus, aneurysm, or orbital damage. These findings were consistent with an unfavorable effect due to compression of the right trigeminal nerve, a condition attributable to a loop in the right superior cerebellar artery (Figures 1 and 2).

The patient returned to the clinic 3 weeks after her initial visit to discuss the results of her imaging study, and at that time she reported significant benefit from the carbamazepine. The patient was advised to continue taking her carbamazepine for another 3 weeks, and after the 6-week course, the medication was tapered to see if discontinuation of carbamazepine would lead to a flare in her trigeminal neuralgia.

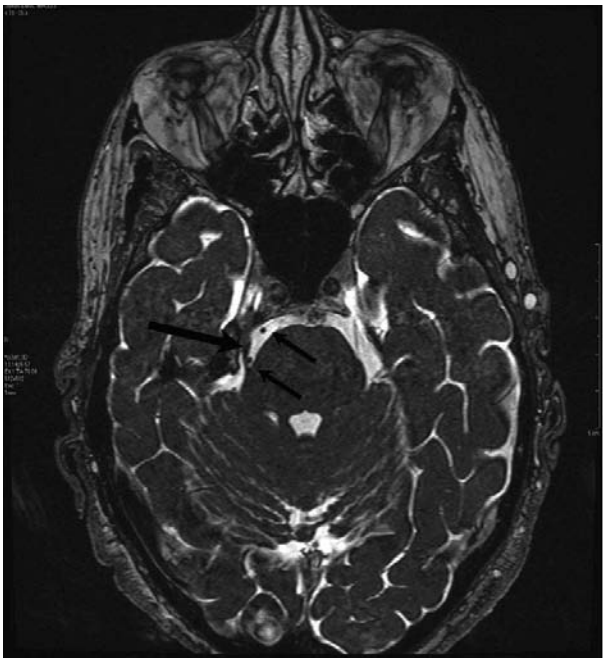

Figure 1. Magnetic resonance image displaying constructive interference in steady-state sequence (3-dimensional volumetric thin slice, T2 weighted) through the level of the mid-pons demonstrates a loop of the right superior cerebellar artery (small arrows), the lower limb of which abuts the right trigeminal nerve (large arrow) as it exits the pons.

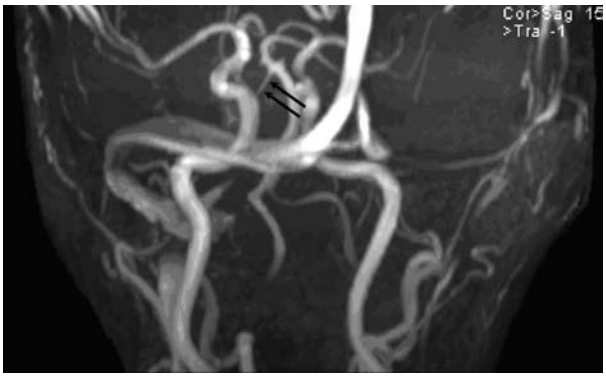

Figure 2.– Oblique view of a 3D time-of-flight magnetic resonance angiogram demonstrating the descending loop of the right superior cerebellar artery (arrows) as described in Figure 1.

DISCUSSION

The trigeminal nerve is the fifth and largest cranial nerve, and it provides cutaneous sensation to the face and innervates the muscles of mastication. The trigeminal nerve exits the brainstem lateral to the pons and rostral to the cerebellum and traverses the cerebellopontine angle cistern before forming the trigeminal ganglion. From this ganglion, the nerve splits into 3 divisions: ophthalmic (V1), maxillary (V2), and mandibular (V3). The delicate anatomy of the posterior fossa places the trigeminal nerve close to several arteries in the cerebellopontine angle cistern. Occasionally anatomical variation of these arteries leads to compression of the nerve. Neurovascular compression has become accepted as the most common cause of trigeminal neuralgia, causing 80%-90% of cases.[6] The vessels that most commonly compress the trigeminal nerve are the superior cerebellar artery and vein.[7] The constant pulsation of the artery against the nerve causes irritation and eventually leads to focal demyelination.[6] Symptoms usually are present in the V2 and V3 distributions of the trigeminal nerve, with solitary involvement of the V1 division, as present in our patient, occurring in only 2.8% of reported cases.[7]

Diagnosis is based on clinical history, neuroimaging, and electrophysiologic tests. Typical diagnostic signs include paroxysmal attacks of pain lasting from a fraction of a second to 2 minutes, affecting 1 or more divisions of the trigeminal nerve. The pain is usually characterized as intense, sharp, or stabbing and is precipitated by trigger factors.

Neuroimaging can help determine the cause of trigeminal neuralgia. Imaging can reveal contributing conditions such as nerve impingement by a tumor and nerve demyelination by multiple sclerosis. Additionally, high-resolution MRI and magnetic resonance angiography allow evaluation for neurovascular compression. Electromyographic studies can also be used to evaluate the blink reflex in response to electrical stimulation of the V1, V2, and V3 distributions to help distinguish primary trigeminal neuralgia from a secondary cause.[10]

The treatments available for trigeminal neuralgia include medical and surgical options. Overall, medical management predominates, and anticonvulsants are the class of medications most frequently used. Carbamazepine remains the standard firstline treatment and has been shown in at least 4 randomized clinical trials to decrease both the frequency and intensity of symptomatic episodes.[11] Although carbamazepine has been estimated to be effective in up to 90% of patients,[11] this drug is not always well tolerated. Gabapentin is also widely used in trigeminal neuralgia despite the fact that all existing literature on its effectiveness is based on case reports and off-label studies.[12] A

large open-label retrospective study by Cheshire in 2002 showed gabapentin to be effective for alleviating the symptoms of trigeminal neuralgia in 43% of cases.[13] Oxcarbazepine has also been used to treat trigeminal neuralgia and has been shown to be as effective as carbamazepine, with a significantly lower incidence of adverse side effects such as vertigo, dizziness, ataxia, and fatigue.[11] Spontaneous remission of symptoms is characteristic of the disease, so regardless of the treatment chosen, a slow taper of medication should be considered in all patients who become asymptomatic.[14]

Successful surgical treatment of trigeminal neuralgia was first performed by Peter Janetta, who cured the disease by microvascular decompression of the trigeminal nerve.[3] This procedure was initially greeted with much skepticism but has gained acceptance based on a large body of evidence demonstrating long-term effective treatment. A large retrospective study of outcomes from the procedure revealed that 82% of patients had excellent immediate postoperative relief and 64% continued to have excellent results 10 years postoperatively.[15] Percutaneous interventions are also available and include radiofrequency thermocoagulation, retrogasserian glycerol injection, and trigeminal ganglion balloon compression.[16,17] Although these procedures are less invasive, all may lead to permanent damage to the trigeminal ganglion and have a high risk of producing neurological problems including facial numbness and paresthesias.[14] Gamma knife therapy without MRI guidance was tried in the 1950s and was abandoned after poor results. Given the new era of MRI-guided gamma knife procedures, however, this method is being revived as an option for treatment. A large prospective trial by Regis et al in 2006 produced initial pain relief in 94% of patients, and this method is a very promising for future treatments.[18]

The patient we describe had trigeminal neuralgia localized to the ophthalmic branch (V1) area of innervation, with more intense pain directly over the supraorbital foramen. This pain pattern is a rare presentation of neurovascular compressive trigeminal neuralgia and is more commonly seen in patients with a history of postherpetic neuralgia. In our patient, however, compression of the trigeminal nerve root by the superior cerebellar artery was confirmed by imaging. Careful history-taking and thorough examination of imaging studies can help clinicians to identify trigeminal neuralgia with an unusual presentation such as the case we describe. Medical management of our patient with carbamazepine provided significant, but not total, symptomatic relief. In patients who become asymptomatic while taking medication, a subsequent taper may reveal a medical "cure." If serious or debilitating symptoms persist with medical management, surgical or gamma knife treatment may be curative, albeit with the added risks of surgery.

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Sickle Cell Disease: An Overview and Translational Research Relevant to Identification of Biomarkers of Pulmonary Inflammation and Fibrosis

Joshua J. Field, MD, Assistant Professor, Department of Medicine, Division of Hematology, Washington University School of Medicine, St. Louis, MO
Robert M. Strieter, MD, Professor of Medicine, Division of Pulmonary and Critical Care Medicine
Joel Linden, PhD, Professor of Medicine, Division of Cardiovascular Medicine

Sickle cell disease (SCD) is the most common inherited disorder in African Americans. SCD includes a group of genetic conditions resulting from autosomal recessive inheritance of a mutated β-globulin gene. The most common of these conditions is sickle cell anemia (SCA), which is due to homozygous inheritance of hemoglobin (Hb) SS. SCA accounts for approximately 70% of cases of SCD. In contrast, the heterozygous (HbAS) state results in a benign carrier state, sickle cell trait. The estimated incidence rates of HbSS and HbAS are 0.14% and 8.6%, respectively, in the African-American population, which predicts that 70,000 African Americans in the United States have SCA. During the last several decades the outlook for individuals with SCD has improved so that more than 90% of individuals born with SCA now live to a mean age of 42 and 48 years for males and females, respectively. The improvement in survival has been multifactorial. Although there has been an increase in mean survival, patients with SCD have experienced an increased frequency of chronic damage to lungs and other organs. In fact, there were more than 110,000 hospitalizations related to SCD in 2004; 75% of these hospitalized patients were adults, and their treatment incurred more than $500 million in health care expenses in the United States alone. It is increasingly apparent that both acute and chronic complications of SCD are common, but these problems are often not fully appreciated by the clinician, partly because organ damage can occur without obvious clinical indications such as severe pain. Therefore, we need additional clinical tools, such as a biomarkers, to assess the subclinical and clinical progression of SCD to chronic end-organ injury, such as chronic SC lung disease. In this Medical Grand Rounds, we review and highlight the importance of translational research relevant to SCD and the discovery of novel biomarkers that may enable clinicians to detect subclinical disease progression in patients with SCD.

INTRODUCTION

Sickle cell disease (SCD), one of the most common genetic diseases in the United States, affects approximately 1 in 400 African American births.[1] The basis for SCD is a single nucleotide substitution in the sixth codon of the β-globin gene. Under hypoxic conditions, the resulting sickle hemoglobin ($\alpha_2 s_2$) polymerizes and changes the conformation of deformable, oval erythrocytes into rigid, sickle-shaped erythrocytes. When transversing the microvasculature, sickle erythrocytes interact with white blood cells, platelets, nonsickle erythrocytes, and endothelial cells, causing vascular occlusion and ultimately tissue ischemia.[2,3] The clinical course of SCD is punctuated by episodes of vasoocclusive crisis and acute chest syndrome (ACS).[4,5] Previously, SCD-related end-organ damage was thought to be the cumulative result of these acute exacerbations of vasoocclusion.[6] Newer evidence, however, has added complexity to the premise that vasoocclusion occurs episodically and the resulting tissue damage is due solely to sickle cells obstructing the microvasculature. The emerging paradigms for the pathogenesis of vasoocclusion are based on the recognition that subclinical vasoocclusive events are ongoing and constant and may significantly contribute to organ dysfunction, and that ischemia-reperfusion injury perpetuates the persistent microvascular inflammatory state, which in turn propagates and sustains vasoocclusive events.

Here we examine this new paradigm of vasoocclusion and focus on the contribution of 2 unique inflammatory cells, fibrocytes and natural killer T (NKT) cells, to pulmonary complications in SCD. We begin with an overview of the clinical features of acute and chronic pulmonary disease in children and adults with SCD. Then we examine the potential role of fibrocytes, bone marrow–derived mesenchymal progenitor cells, in the pathogenesis of chronic sickle cell lung disease (CSCLD).

Finally, we discuss ischemia-reperfusion injury and the important role of NKT cells in promoting vasoocclusion and lung injury.

Pulmonary Function in Children and Adults with SCD

Pulmonary complications are a leading cause of morbidity and mortality in children and adults with SCD.[7,8] Acute pulmonary complications, such as ACS and asthma, are more common in children with SCD, whereas chronic lung disease predominates in adults.[5,9] ACS is the most common pulmonary complication in SCD, affecting approximately 50% of individuals during their lifetime.[10] ACS is defined as a new pulmonary infiltrate visible on chest radiograph, but ACS patients may also present with chest pain, fever, hypoxemia, and respiratory symptoms.[7] Many processes may contribute to ACS, including infections, fat emboli, and atelectasis; however, vasoocclusion, systemically and within the pulmonary vasculature, is the cornerstone of ACS pathogenesis.[7-11] Consequently, ACS episodes are typically accompanied by increased hemolysis and decreasing hemoglobin.[11] Because ACS is a common complication in children and adults with SCD, several studies have evaluated the impact of ACS episodes on pulmonary function and the development of CSCLD.

Children with SCD frequently have abnormal pulmonary function, most commonly with an obstructive pattern.[12-15]. Sylvester and colleagues examined 64 children with SCD to determine if lung function in children with SCD differed significantly from that in children in the general population.[12] Forced expiratory volume in 1 second and forced vital capacity were significantly lower in children with SCD compared to age- and race-matched controls. Abnormalities of pulmonary function were increasingly more common with age. Consistent with this finding, a study of 79 children with SCD revealed that growth of lung function was less in children with SCD compared to children in the general population.[16] Findings have been inconsistent regarding the relationship between obstructive lung disease and ACS in children with SCD. A study of 63 children with SCD who underwent pulmonary function testing found obstructive lung disease in 35% of children examined, whereas restrictive defects were noted in only 8%.[15] There was no association of prior episodes of ACS or a history of asthma with obstructive lung disease. However, a subsequent case-control study of 40 children with and without a history of ACS episodes showed increased airway obstruction in children with SCD and a history of ACS compared to those without ACS.[13]

In contrast to children with SCD, adults with SCD frequently have pulmonary fibrosis, and pulmonary function testing frequently shows a restrictive pattern.[6,17,18] CSCLD is characterized by increased interstitial markings visible on chest radiograph, dyspnea, pulmonary hypertension, and restrictive defects on pulmonary function testing.[6] Although CSCLD affects a minority of adults with SCD, it is associated with increased mortality, with a mean time to death following diagnosis of 5.2 years.[6] The strongest predictor of CSCLD is the number of prior episodes of ACS.[6] Although many adults with SCD may not exhibit all of the characteristics of CSCLD, a restrictive pattern on pulmonary function testing is prevalent. According to data from the Cooperative Study of Sickle Cell Disease (n = 310) reported by Klings and associates, 74% of adults with SCD had restrictive lung disease.[17] Risks for a restrictive pattern included lower hemoglobin (Hb) and higher white blood cell count, known markers of disease severity. Notably, the number of prior episodes of ACS was not associated with restricted lung disease (P = .06). Selection bias may have limited the generalizability of this study's findings, given that the original study population comprised 2061 individuals with SCD, yet only 310 adults were included in the final analysis. Smaller studies of pulmonary function in adults with SCD, however, have confirmed the presence of restrictive lung disease.[18,19]

Pulmonary hypertension, defined as a tricuspid regurgitant jet velocity ≥2.5 m/sec, is an independent risk indicator for mortality in adults with SCD,[11] and restrictive lung disease may contribute to its development.[19] Anthi and colleagues, who examined 43 adults with SCD, reported a significant relationship between tricuspid regurgitant jet velocity and lower forced vital capacity, indicating a trend toward restrictive lung disease.[19] Furthermore, patients with evidence of increased interstitial changes on lung computed tomographic scan had a higher tricuspid regurgitant jet velocity compared to patients with normal computed tomographic findings, although this result did not achieve statistical significance. Taken together, these studies demonstrate that restrictive lung disease is a common problem for

adults with SCD and may contribute to increased mortality through the development of CSCLD and/or pulmonary hypertension.[20]

Despite the prevalence of acute and chronic lung disease among children and adults with SCD, the mechanism for these abnormalities is not well defined. Clearly, there is an evolution of lung function abnormalities from an obstructed to restricted pattern as individuals with SCD age. Repetitive infarcts occurring during ACS episodes do not adequately explain this pulmonary dysfunction. The pulmonary fibrosis characteristic of CSCLD is similar to that seen in disorders that affect the lung in patients without SCD, including idiopathic pulmonary fibrosis (IPF) and other interstitial lung diseases. Recent studies have implicated fibrocytes, circulating bone marrow-derived mesenchymal progenitor cells, in the pathogenesis of pulmonary fibrosis.[21,22]. Fibrocytes released from the bone marrow in response to vasoocclusive-induced tissue damage may contribute to the pulmonary function abnormalities and chronic restrictive lung disease common in SCD.

Origin of the Fibroblast/Myofibroblast: A Pivotal Cell in Mediating Fibroproliferation in Pulmonary Fibrosis

The fibroblast/myofibroblast plays a pivotal role for the generation of the extracellular matrix (ECM) in response to tissue injury and fibrosis. Understanding the origin of these cells is critical to understanding their biology in this process. Currently, 1 classical and 2 contemporary theories exist that fit the origin of fibroblasts/myofibroblasts in lung tissue during the pathogenesis of pulmonary fibrosis.[23-28] The classical concept is that tissue injury induces activation of a resident interstitial fibroblast to differentiate into a myofibroblast that migrates into the intraalveolar space, proliferates, and expresses constituents of the ECM leading to intraalveolar and interstitial pulmonary fibrosis.[26-28] One contemporary concept is that lung injury and changes in the microenvironment of the epithelium, including the basement membrane, can induce epithelial cells to transition to a mesenchymal phenotype, to become fibroblasts/myofibroblasts, and these cells subsequently contribute to fibroproliferation.[24,28] A second contemporary paradigm is that circulating cells, termed fibrocytes, which may be derived from bone marrow precursor cells, can home and extravasate into sites of tissue injury under specific guidance by chemoattractants (ie, chemokines), differentiate into myofibroblasts, proliferate, and contribute to the generation of ECM relevant to pulmonary fibrosis.[22,23,25,29-34]

The Role of Fibrocytes in Human Pulmonary Fibrosis

Given the role of the chemokine biological axis CXCL12/CXCR4 in recruiting fibrocytes to the lung in animal models of pulmonary fibrosis; studies have been extended to patients with pulmonary fibrosis (idiopathic nonspecific interstitial pneumonia [NSIP] and IPF) to better delineate whether fibrocytes are present in human pulmonary fibrosis.[35,36] Immunohistochemical analysis has revealed extensive accumulation of the CXC chemokine, CXCL12, in lung tissue from patients with a clinical and histologic diagnosis of IPF that was associated predominantly with hyperplastic type II pneumocytes. Proteomic analysis revealed that the lungs of patients with fibrotic NSIP and IPF had increased levels of CXCL12 compared to lungs of healthy individuals.[35] To determine whether the increased expression of lung CXCL12 in patients with fibrotic lung disease was correlated with fibrocyte trafficking, a pilot study was performed on prospectively collected peripheral blood samples from 5 patients with fibrotic NSIP and IPF and 5 healthy volunteers.[35] Plasma CXCL12 levels were compared between the groups, and plasma CXCL12 levels were found to be 2.4-fold higher in patients with fibrotic NSIP and IPF than in healthy individuals.[35] These investigators enumerated fibrocytes in peripheral blood by use of quantitative fluorescence-activated cell sorting (FACS) and compared the number of circulating fibrocytes in the 2 groups of pulmonary fibrosis patients. Compared to healthy volunteers, patients with fibrotic lung disease had an order of magnitude higher number of circulating fibrocytes. Fibrocytes, which normally constitute approximately 0.5% to 1% of circulating leukocytes,[22,25,37] comprised 6%-10% of leukocytes in patients with fibrotic lung disease.[35] The expanded circulating fibrocytes in these patients were most notable to be the $CXCR4^+$ subset. In addition, the increase in circulating fibrocytes correlated with the presence of peripheral blood monocytosis in these patients.[35] The finding of peripheral blood monocytosis is relevant to SCD because patients with SCD who experience homeostatic or vasoocclusion events have been reported to have an absolute peripheral blood monocytosis.[37] Because circulating fibrocytes can differentiating into αSMA^+ cells both in vitro

and in vivo,[23,38] the degree of differentiation of the circulating fibrocytes in 2 groups of patients with fibrotic lung disorders was analyzed by FACS analysis to assess the expression of αSMA. The αSMA-expressing fibrocytes constituted a smaller subset of total circulating fibrocytes in both groups. Although no statistically significant differences were found between patients and healthy volunteers for total αSMA^+ fibrocytes or the $CXCR4^+$ or $CXCR4^-$ subsets of αSMA^+ fibrocytes, there appeared to be a significant trend toward higher numbers of circulating αSMA^+ cells in patients with fibrotic interstitial lung diseases. These findings suggest that greater numbers of circulating fibrocytes are present in patients with fibrotic interstitial lung diseases, and these cells may show early evidence of differentiation to a myofibroblast phenotype.

These findings have been further supported by Andersson-Sjoland and colleagues, who recently demonstrated the presence of fibrocytes in lung tissue of patients with IPF.[39] These investigators determined that combinations of CXCR4 and a mesenchymal marker (ie, αSMA) showed staining of significantly more fibrocytes per square millimeter of tissue compared with combinations using CD34 or CD45RO and a mesenchymal marker.[39] Andersson-Sjoland et al also observed a positive correlation between the abundance of fibroblastic foci and the number of lung tissue fibrocytes.[39] No fibrocytes were identified in normal lung tissue. CXCL12 was found to be increased in plasma of patients with IPF and was detectable in the bronchoalveolar lavage fluid of 40% of the patients but not in fluid from controls.[39] Andersson-Sjoland et al determined that the cellular source of CXCL12 in the lung was alveolar type II pneumocytes, and these investigators found that with exercise there was a negative correlation between plasma levels of CXCL12 with DLco (diffusion lung capacity for carbon monoxide) and oxygen saturation.[39] Although the above studies supported the notion that fibrocytes were present in the circulation and in the lungs of patients with IPF, these studies did not look at whether these cells could serve as biomarkers for prognosis in these patients. Recently, Moeller and associates[21] found that the magnitude of circulating fibrocytes was an independent predictor of early mortality of IPF patients and was a better indicator of poor prognosis in these patients than forced vital capacity, DLco, radiological severity scores, or 6-minute walk test. Taken together, the results of the above studies support the notion that circulating fibrocytes, recruited through the CXCL12/CXCR4 biological axis, contribute to the expansion of the fibroblast/myofibroblast population in patients with pulmonary fibrosis, and the magnitude of circulating levels of these cells appears to directly correlate with prognosis in these patients. Thus, understanding the biology of fibrocytes in SCD may lead to a paradigm shift in our knowledge regarding their role in mediating the pathogenesis of pulmonary fibrosis relevant to the development of CSCLD in patients with SCD.

Murine Model of SCD Demonstrates Significant Lung Injury under Homeostatic Conditions

The development of an animal model of SCD is critical to further our understanding of the role of fibrocytes in mediating the pathogenesis of pulmonary fibrosis in CSCLD, to test hypotheses relevant to the pathogenesis of CSCLD, and to allow the assessment of innovative therapeutic interventions. On this basis, we initiated studies with NY1DD mice (previously known as NY-S or NY-einstein mice; congenic with C57BL/6 mice), a gift from R. Hebbel, University of Minnesota Medical School, Minneapolis, MN. The NY1DD mice ($\alpha^H\beta^S[\beta^{MDD}]$) are homozygous for a spontaneous deletion of the mouse β^{major}-globin locus (β^{MDD}) and have a human α- and β^S-globin transgene ($\alpha^H\beta^S$). These mice express approximately 75% human β^s of all β-globins (human β^s-globin forms symmetrical tetramers with human α-globin [approximately 42%] and with mouse α-globin [approximately 30%]).[40-42] The NY1DD mice, compared to other human transgenic sickle cell mice, show milder pathology, yet can exhibit multiple organ damage.[40-42] We initially characterized the differential of peripheral blood leukocytes in the NY1DD mice compared to wild-type C57BL/6 controls under homeostatic conditions. The NY1DD mice at age 6 to 8 weeks had mild reductions in their Hb, compared to controls under steady-state conditions. In contrast, the NY1DD mice demonstrated elevated leukocyte counts in their peripheral blood under homeostatic conditions that were indicated by increased neutrophils, monocytes, and lymphocytes. Assessment of end organs in the NY1DD mice under homeostatic conditions at age 6 to 8 weeks revealed significant gross pathologic injury in lungs, spleen, kidney, and liver. Histological examination of the lungs of the NY1DD mice compared with lungs of control mice revealed an increase in leukocyte infiltration, with alveolar septal thickening and evidence for

intraalveolar hemorrhage with proteinaceaous deposition. These findings indicated the presence in NY1DD mice of hypoxemia, increased vascular permeability, increased respiratory rate, and reduced tidal volume compared to control mice. Taken together, the above findings in 6- to 8-week-old NY1DD mice during homeostatic conditions demonstrated marked lung pathology and pathophysiology relevant to adult SCD patients with subclinical and clinical lung injury.

NY1DD SCD Mice Demonstrated Increased Deposition of ECM in their Lungs under Homeostatic Conditions

Because the NY1DD mice displayed marked lung injury under homeostatic conditions, we next searched for evidence for a fibroproliferative phase of lung injury. Using the Sircol assay as previously described [23,43] to measure total soluble collagen in the lungs of NY1DD mice at 6 to 8 weeks, we found increased levels of soluble collagen in the lungs of these mice compared to control mice. We confirmed these findings by morphometric analysis of the lungs with the collagen-specific dye picrosirius red. To put into context the levels of soluble collagen in the lungs of these SCD mice, we compared these levels to those in C57BL/6 mice that had been exposed to bleomycin for 16 days. Levels of soluble collagen in the lungs of mice exposed to bleomycin were similar to the levels of soluble collagen in the lungs of NY1DD mice that had been maintained under homeostatic conditions. Thus in the lungs of NY1DD mice compared to control mice we observed active fibroproliferation, a finding that may be relevant to lungs of SCD patients under homeostatic conditions as well as in the presence of vasoocclusive events, ACS, and CSCLD.

Fibrocytes Were Increased in Bone Marrow, Circulation, and Lungs of NY1DD SCD Mice under Homeostatic Conditions

To determine whether the pathogenesis of increased fibroproliferation in the lungs of the NY1DD mice was attributable to the presence of fibrocytes, we next assessed the quantitative levels of fibrocytes in the bone marrow, circulation, and lungs of these mice compared to controls. Numbers of fibrocytes were found to be markedly increased in the bone marrow, circulation, and lungs of these mice. The chemokine-receptor hierarchy that was found expressed on fibrocytes in the bone marrow, circulation, and lung demonstrated the expression pattern of CXCR4>>CCR2>CCR7. To further confirm that these were fibrocytes and not macrophages that had phagocytized collagen, we performed FACS analysis on these cells to detect intracellular pro-collagen type I and III by using specific antibodies that detect the C-terminus and N-terminus of type I pro-collagen and the C-terminus of pro-collagen III. Fibrocytes that were positive for pro-collagen type I and III were found to be increased in the bone marrow, circulation, and lungs of NY1DD mice. In addition, we found increased numbers of fibrocytes that appeared to be undergoing differentiation to αSMA^{+} cells in the bone marrow, circulation, and lungs of NY1DD SCD mice. These findings support the notion that the fibrocyte population is expanded in the bone marrow, from which the fibrocytes are mobilized to the circulation and then home to and extravasate in the lung. Moreover, on these fibrocytes there appears to be a chemokine receptor hierarchy of expression with a pattern of CXCR4>>CCR2>CCR7, which suggests that CXCR4 may be important for their homing and extravasation into the lung of NY1DD mice.

NY1DD SCD Mice Display Increased Levels of CXCL12 and Other Cytokines in their Lungs under Homeostatic Conditions that Are Relevant to Fibrocyte Biology

After we demonstrated the presence of increased fibrocytes in the lungs of these mice, we next determined whether CXCL12 was elevated in the lungs of NY1DD mice under homeostatic conditions. Luminex protein multiplexing of lung homogenates, performed as previously described,[23] revealed that CXCL12 was markedly elevated in the lungs of NY1DD mice and that the levels of CXCL12 were similar to those that we had found in the lungs of bleomycin-exposed mice at day 8 to 20 and correlated with maximal ECM deposition in this mouse model.[23] Moreover, when we searched for other cytokines that might be relevant to fibrocyte biology, we found elevated levels of platelet-derive growth factor (PDGF) and macrophage colony-stimulating factor (M-CSF) in the lungs of these animals. PDGF activation may be important in proliferation of fibrocytes, as well as in signaling events relevant to expression of chemokine receptors. Our results indicate that the presence of M-CSF in the lung and plasma of these mice under homeostatic conditions is extremely relevant to fibrocyte behavior, because we found that systemically administered M-CSF in wild-type mice leads to expansion of fibrocyte populations in

the bone marrow and mobilization of fibrocyte cells into the circulation.

Depletion of CXCL12 under Homeostatic Conditions IN NY1DD Mice Resulted in Marked Attenuation of Extravasated Fibrocytes into their Lungs that Correlated with a Significant Reduction in Deposition of Collagen

On the basis of the presence of CXCL12 in the lungs of NY1DD mice under homeostatic conditions that directly correlated with the presence of increased numbers of $CXCR4^+$ fibrocytes, we next examined whether depletion of CXCL12 could have an impact on extravasation of fibrocytes into the lungs of NY1DD mice, and whether this would have a direct effect on the magnitude of collagen deposition. NY1DD mice under homeostatic conditions were treated with neutralizing anti-CXCL12 or control antibodies for a period of 7 days. Then the mice were killed, and we used quantitative FACS to assess their lungs for levels of fibrocytes and soluble collagen measurement to assess ECM deposition. Results demonstrated a marked attenuation of the presence of $CXCR4^+$ fibrocytes in the lungs of the NY1DD mice that had been depleted of CXCL12, findings that directly correlated with a reduction in soluble collagen. Moreover, when we assessed the impact of this therapeutic strategy on other populations of leukocytes in the lungs of these mice, we found no significant impact on CD4, CD8, NK cells, neutrophils, or monocytes. These findings suggest that under homeostatic conditions the lungs of NY1DD mice are undergoing collagen deposition that appears to be related to extravasation of $CXCR4^+$ fibrocytes into their lungs. Furthermore, depletion of CXCL12 did not perturb other leukocyte populations that were found in the lungs of these mice. Furthermore, these findings were similar to those we had reported in the model of bleomycin-induced pulmonary fibrosis.[23]

Fibrocytes Were Markedly Elevated and Activated in the Circulation of Patients with SCD

Because our studies in preclinical animal models of SCD relevant to patients with SCD demonstrated that fibrocyte cells may be major contributors to the pathogenesis of the fibroproliferative phase of lung injury under homeostatic conditions, we next determined whether fibrocytes were present in patients with SCD under similar conditions (i.e., no clinical evidence of vasoocclusive events or ACS). The patients with SCD (10 females, average age 32 years, and 9 males, average age 30 years), compared to 4 age-matched healthy individuals, were defined as clinically stable, without evidence for any acute exacerbation. We used quantitative FACS analysis to assess de novo the circulating levels of fibrocytes in these patients. FACS analysis of the buffy-coat cells from these patients demonstrated markedly elevated levels of fibrocytes in their circulation, compared to that of age-matched controls. When we assessed the hierarchy of chemokine-receptor expression on these fibrocytes, we found the following pattern of expression: CXCR4>>CCR2>CCR7. These results were in keeping with our previous findings of CXCR4 predominance on circulating fibrocytes in patients with pulmonary fibrosis.[35] Furthermore, as we had seen in our transgenic SCD mice, we found markedly elevated plasma levels of CXCL12, M-CSF, and PDGF in patients with SCD, cytokines relevant to fibrocyte biology.

Because fibrocyte cells are progenitors to αSMA^+ myofibroblastlike cells, we next used quantitative FACS analysis to ascertain whether the elevation in circulating fibrocytes in the SCD patients was associated with increased numbers of αSMA^+ fibrocytes.[20,35]. We determined that SCD patients demonstrated a significant increase in circulating αSMA^+ fibrocytes. We had previously determined that transforming growth factor β(TGF-β) stimulation of fibrocytes via activation of receptor Smads (Smad2/3) was critical to initiate signal transduction and induction of αSMA in these cells.[38] On this basis, we developed a strategy in our laboratory to detect activated receptor Smads (pSmad2/3) by quantitative FACS analysis, which we confirmed by using Western blot analysis of cells stimulated with TGF-β. Fibrocytes in the circulation of patients with SCD displayed markedly elevated activated receptor Smads (pSmad2/3). These findings suggested that these cells were activated by TGF-β either while they were in circulation or prior to mobilization in the bone marrow. This process may represent a mechanism by which circulating fibrocytes differentiate to αSMA^+ cells in these patients. These studies demonstrate that bone marrow–derived fibrocytes are found markedly elevated in the bone marrow, circulation, and lungs in a preclinical animal model of SCD and in the circulation of patients with SCD. These findings support the notion that fibrocytes may be a causative cell in the pathogenesis of CSCLD and may be a novel biomarker for monitoring subclinical

and clinical disease progression in patients with SCD.

The Role of Ischemia-Reperfusion Injury in Promoting Lung Injury in SCD

Because the pulmonary arterial circulation has low oxygen tension and pressure and low blood velocity and constricts in response to hypoxia, the lung microenvironment is particularly conducive to the polymerization of HbS and is therefore highly vulnerable to ischemia-reperfusion injury.[44] Consequently, pulmonary disease is the leading cause of morbidity and mortality in patients with SCD.[11, 45]

The hallmark of SCD in patients is vasoocclusive crisis. Recent findings suggest that transient microvascular occlusion occurs chronically in a subclinical manner in SCD patients, and that end-organ damage and short lifespan in SCD patients are due to the cumulative effects of repeated bouts of ischemic events.[46,47] Historically, microvascular occlusion was thought to be caused by rigid sickled erythrocytes. More recently it has been noted that vasoocclusive crisis is associated with a proinflammatory, procoagulatory, and proadhesive hemodynamic phenotype in SCD that involves interactions between leukocytes, red blood cells, vascular endothelial cells, and platelets that contribute to microvascular inflammation and injury.[48,49]

NKT Cells Mediate Ischemia-Reperfusion Injury in the SCD Lung

NKT cells have recently been shown to contribute to the pathology of pulmonary, hepatic, cardiac, and renal ischemia-reperfusion injury.[50-53] In healthy individuals, NKT cells comprise a relatively minor subset of lymphocytes (approximately 0.5% of the T-cell population in the blood and peripheral lymph nodes, approximately 2.5% of T cells in the spleen, mesenteric, and pancreatic lymph nodes, and up to 30% of T cells in the liver).[54] Invariant NKT (iNKT) cells, also known as type I, express a restricted T-cell receptor (TCR) (Vα14-Jα18 [murine] or Vα24-Jα18 [human]) that is activated by lipid antigen presentation by CD1d (a major histocompatibility complex I–like molecule) found on antigen-presenting cells.[55,56] CD1d activation has been hypothesized to occur as a result of presentation of host lipids that are byproducts of the degradation of necrotic or apoptotic cells.[57] Type II NKT cells express more diverse lipid-binding TCRs and are CD1d restricted but unresponsive to α-GalCer; type III NKT cells have diverse TCRs.[58]

On TCR activation, iNKT cells rapidly release large quantities of cytokines including interleukin-2 (IL-2), IL-4, and interferon γ (IFN-γ), which promote activation of dendritic cells, NK cells, B cells, and conventional $CD4^+$ and $CD8^+$ T cells.[59-62]. Furthermore, IFN-γ can stimulate endothelial, epithelial, neuronal, and lymphoid cells to release IFN-γ–inducible CXC chemokines (CXCL9, CXCL10, and CXCL11), which are potent chemoattractants for mononuclear cells that express CXCR3 (such as activated T cells and NK cells).[63-66]

The rapidity of reperfusion injury is not consistent with the timeframe required for activation and differentiation of conventional $CD4^+$ T-cell responses, suggesting that injury is mediated by a rapidly activated T-cell subset. This theory prompted us to investigate NKT cells. The majority of mouse $CD4^+$ $NK1.1^+$ NKT cells express the invariant TCR Vα14Jα18 and are dependent on CD1d for positive selection in the thymus and subsequent activation in the periphery.[54,67] CD1d is expressed by antigen-presenting cells and also by hepatocytes, gut epithelial cells, and certain B cells and monocytes. CD1d presents unidentified self-lipid antigens, microbial glycolipids, or α-Gal-Cer,[68] a glypolipid that is derived from a marine sponge, to invariant TCRs on NKT cells. The rapid release of IFN-γ, IL-2, and IL-4 following activation of iNKT cells by CD1d-glycolipid presentation to TCRs has been attributed to preformed cytokine transcripts.[69] This rapid response of iNKT cells to activation suggests that they might play a role in reperfusion injury. We first demonstrated this role in liver reperfusion injury. Depletion of NKT and NK cells with PK136, an antibody that binds to NK1.1 and is found only on NKT and NK cells, or blockade of CD1d-restricted iNKT cell activation with an anti-CD1d antibody produced significant protection from liver ischemia-reperfusion injury that is equivalent to and not additive with protection by the $A_{2A}R$ agonist ATL146e. In addition, we demonstrated that IFN-γ production by activated NKT cells isolated from spleen is potently inhibited by ATL146e, and this inhibition is competitively blocked by the selective $A_{2A}R$ antagonist ZM241385.[52]

In NY1DD mice as a mouse model of SCD, we used fluorescent CD1d tetramers, loaded with the α-GalCer–like glycolipid PBS57, to selectively label

pulmonary iNKT cells (defined as tetramer$^+$ CD3$^+$). FACS analysis and counting beads were used to determine the absolute numbers of iNKT cells and the percentage of iNKT cells among all lymphocytes (live, CD45$^+$ low side-scatter cells) in lungs from NY1DD and congenic wild-type C57BL/6 mice. Relative to wild-type mice, pulmonary iNKT cells in NY1DD mice were increased in absolute number from $2.4 \pm 0.3 \times 10^4$ to $4.3 \pm 0.4 \times 10^4$ and as a percentage of all lymphocytes from 1.4% ± 0.09% to 3.6% ± 0.5%. To determine the activation state of pulmonary iNKT cells, we analyzed surface expression of CD69 and intracellular IFN-γ, which are both well characterized markers of iNKT-cell activation.[70] NY1DD mice displayed significantly increased levels of both markers compared to C57BL/6 mice. The percentage of pulmonary iNKT cells positive for IFN-γ increased significantly from 5% in wild-type mice to 37% in NY1DD mice, a 7.4-fold difference.

Blocking of CD1d-Restricted iNKT Cell Activation in NY1DD Mice Reduces Lung Inflammation and Injury

To determine if iNKT cells are involved in pulmonary injury, NY1DD mice were treated on 2 consecutive days with anti-CD1d antibody (or isotype control antibody) to inhibit CD1d-restricted activation of iNKT cells. One treatment group was assessed the day after the second anti-CD1d injection, at the peak of the antibody response, and another treatment group was assessed 4 days later (5 days after antibody treatment), when the antibody response had dissipated. One day after treatment of NY1DD mice with anti-CD1d, we noted a significant decrease in vascular permeability, a significant increase in arterial oxygen saturation, and significantly normalized breathing parameters. All of these effects were largely or completely reversed 5 days after antibody treatment. We also found that the effects of anti-CD1d antibody treatment to protect the lung were replicated by depleting NKT and NK cells with anti-NK1.1 antibodies.

Expansion and Activation of iNKT Cells from SCD Patients

In an investigation similar to our study of circulating fibrocytes in patients with SCD, we determined whether iNKT cells were present in patients with SCD under similar conditions (i.e., no clinical evidence of vasoocclusive events or ACS). The patients with SCD (10 females, average age 32 years, and 9 males, average age 30 years), compared to 4 age-matched healthy individuals, were defined as clinically stable, without evidence for any acute exacerbation. We used quantitative FACS analysis for de novo assessment of the circulating levels of iNKT cells in these patients. iNKT cells in human blood were detected based on binding of fluorescent, glypolipid-loaded human CD1d tetramers. iNKT cells in the blood of SCD patients were found to be markedly expanded. Given the general leukocytosis of patients with SCD, it is notable that there is selective expansion of iNKT cells among lymphocytes, from <1% in control blood to an average of 6% in the blood of SCD patients. We also examined the activation state of iNKT cells. SCD causes a significant increase in the percentage of iNKT cells positive for CXCR3 and CD69 and a strong trend toward an increase in intracellular IFN-γ. On the basis of these data we concluded that SCD produces a similar expansion and activation of iNKT cells in mice and SCD patients.

In summary, these translational studies demonstrate the presence of 2 novel biomarkers (fibrocytes and iNKT cells) that are directly involved in the pathogenesis of SCD lung disease, as well as biomarkers that may be used in longitudinal studies to monitor subclinical disease progress and/or to predict vasoocclusive events that contribute to vasoocclusive crisis and evolution of chronic end-organ injury in patients with SCD. On this basis, ongoing longitudinal studies are underway to test these hypotheses in patients with SCD.

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A Case of Being "Double-Positive"

Amy West, MD, Fellow Physician, Department of Medicine, Division of Cardiovascular Medicine
Andrew Bellizzi, MD, Associate Pathologist, Department of Pathology, Brigham and Women's Hospital, Boston, MA, Pathology Instructor, Harvard Medical School, Boston, MA
W. Kline Bolton, MD, Professor of Medicine, Division of Nephrology

CASE PRESENTATION

The patient was a 71-year-old woman who was in her usual state of health until 9 months earlier when she presented to her primary care doctor's office with a suspected viral upper-respiratory infection. She received supportive care and initially noted improvement in her dyspnea and cough. The patient returned 6 months later complaining again of a dry cough. Her only notable medical history at that time was hypertension, for which she was treated with an angiotensin-converting enzyme inhibitor, and hyperlipidemia, treated with a statin. She reported a remote history of tobacco use, no illicit drug use, and consumption of alcohol socially. The results of her physical exam were unremarkable. Laboratory test results included a creatinine of 0.5 mg/dL, and urinalysis was positive for nitrite, leukocyte esterase, and a few bacteria. She was started on trimethoprim-sulfamethoxazole for an uncomplicated urinary tract infection. Her cough was attributed to the angiotensin-converting enzyme inhibitor and resolved promptly after discontinuation of that drug.

While being treated for the urinary tract infection, the patient developed low-grade fevers, and she received a 1-week course of ciprofloxacin because of concern that she might have had a complicated urinary tract infection. Despite this course of antibiotics, the intermittent fevers continued, Urine culture results revealed 20,000 colonies of *Enterococcus faecalis* sensitive to both penicillin and quinolones, and the patient received a 1-week course of amoxicillin-clavulanate.

Despite her treatment with antibiotics, the patient had persistent fevers, and she was admitted to a local hospital for evaluation. Her initial laboratory results revealed a creatinine of 1.6 mg/dL, which rose to 2.3 mg/dL over the next 3 days and ultimately peaked at 2.8 mg/dL. The patient's renal ultrasound revealed normal kidney size, no hydronephrosis, and patent renal vasculature. A urinanalysis showed no infection, but her urine eosinophils were mildly positive at 1%. The blood work showed a white blood cell count of 22,000/mL with 78% polymorphonuclear cells, hematocrit 30%, and platelet count 660,000/mL. Results of her urine culture and 2 sets of blood cultures were negative.

Differential Diagnosis

Given the patient's presentation with renal failure in the setting of recent antibiotic use, acute interstitial nephritis (AIN) must be considered. The finding of eosinophiluria is commonly associated with acute interstitial nephritis. The sensitivity of urinary eosinophils has been estimated at 67% with a specificity of 83%.[1,2] The extrarenal manifestations of acute interstitial nephritis, low-grade fevers, maculopapular rash, arthralgias, and eosinophilia are related to a hypersensitivity reaction and are seen in less than 50% of patients.[3]

In the past, the most common inciting drug for AIN was methicillin, although the spectrum of possible offending antibiotics includes other penicillin-based drugs, sulfonamides, ciprofloxacin, and rifampin.[1] Numerous drugs are associated with AIN as well as multiple bacterial, viral, and fungal infections. The renal damage from drug-induced AIN can occur anywhere from 3 to 5 days after secondary exposure to a medication and up to several weeks after an initial dose of a new drug. The diagnosis of drug-induced AIN is made on the basis of laboratory findings of urinary eosinophils, white blood cell casts, and renal insufficiency and a temporal relationship to an offending medication. The diagnosis of AIN can be aided by the use of a gallium scan, which can show diffuse, intense bilateral uptake of the gallium, with a reported sensitivity of 60%-100%.[4] However, given the limited diagnostic utility of gallium scans, they are rarely used in cases of suspected AIN.[5] A renal biopsy provides confirmation of the diagnosis but is unnecessary in most cases.

The treatment strategy for drug-induced AIN involves discontinuing the offending agent and monitoring for improved creatinine. In a study of 27 patients with biopsy-proven AIN, 63% of

patients demonstrated spontaneous improvement in creatinine during a 2-week period.[6] Prednisone treatment is used but controversy exists regarding both the timing and duration of therapy. Many authors recommend giving a short course of prednisone in patients with drug-induced AIN whose renal function does not improve within 1-2 weeks of discontinuing the offending agent.[1]

Interval History

On the basis of the patient's fevers, eosinophiluria, and recent exposure to ciprofloxacin, the patient was treated for presumed AIN with a 5-day course of prednisone. Initially her creatinine improved from 2.8 mg/dL to 2.3 mg/dL and then remained at 2.4 mg/dL. During the next 5 weeks she had daily fevers to 39°C. Two weeks after completing a course of prednisone she had worsening renal failure with a serum creatinine of 4.4 mg/dL. The differential diagnosis at this point was expanded to include intraabdominal abscess, occult malignancy, and endocarditis. A computed tomographic scan of the chest, abdomen, and pelvis performed without intravenous contrast showed normal anatomy and with no evidence of lymphadenopathy or abscess. She had 5 sets of negative blood cultures and was scheduled to have a transthoracic echocardiogram as an outpatient. For further evaluation of her fevers and renal failure she was admitted to the University of Virginia Health System.

On physical examination at this hospital the patient was in no acute distress. Her temperature was 38°C. Her blood pressure was 147/67 mm Hg and her heart rate 68 beats/min. She was not tachypneic, and her oxygen saturation was 97% on room air. She had crackles at her left base, without egophony, dullness to percussion, or wheezing. Her cardiac exam revealed a regular rate and rhythm, without murmurs or gallops. Her abdomen was without hepatosplenomegaly. She had no palpable lymphadenopathy and no detectable rashes.

The general medicine team was concerned about the worsening of the patient's renal failure in the setting of a fever of unknown origin (FUO).

Differential Diagnosis

FUO was originally described as a clinical entity in 1961[7] by Petersdorf and Beeson as a fever greater than 38.3ºC lasting more than 3 weeks with an unexplained diagnosis despite 1 week of inpatient evaluation. In 1991 a revised set of diagnostic criteria for the classic group of FUO patients were published. The criteria were revised in 1991 by Durak and Street and expanded to allow for an increased role of outpatient evaluation. With the revised criteria for FUO, there must be an evaluation of at least 3 days in the hospital and 3 outpatient visits or 1 week of logical and intensive outpatient investigation.[8] The other significant revision made by Durak and Street was to further categorize potential etiologies of FUO based on patient characteristics: classic, nosocomial, immune deficient, and HIV associated.[9]

The initial evaluation prior to establishment of a diagnosis of FUO includes comprehensive medical history and physical examination and baseline laboratory testing. The diagnostic work-up for FUO includes a complete blood count and differential, blood film, comprehensive chemistry panel, antinuclear antibodies, rheumatoid factor, HIV antibody, and chest x-ray.[10] Any abnormal findings are then evaluated with additional diagnostic studies. Any medications with the potential to cause a drug fever should be discontinued. Once the diagnosis of FUO has been established, further diagnostic testing is performed, beginning with an abdominal computed tomographic scan and evaluation of the patient for the Duke criteria for endocarditis, a process that includes echocardiography, lower extremity venous Doppler studies, and in elderly patients, temporal artery biopsy.[11] There is no clear evidence supporting the use of erythrocyte sedimentation rate (ESR) or C-reactive protein in FUO. However, with the appropriate level of clinical suspicion based on results of the patient's initial evaluation for a potential rheumatologic process, occult infection, or malignancy, a markedly elevated ESR > 100 mm/h can be a helpful diagnostic indicator.[11]

In the patient we describe, a diagnosis of classic FUO was likely based on her history of fevers for more than 3 weeks as an outpatient and her having had an evaluation of at least 3 days in the hospital. A recent systematic review of articles pertaining to FUO and published from 1966 to 2000[12] included 11 case series with more than 1000 patients. The largest categories of conditions underlying FUO included infection (28%), inflammatory diseases (21%), and malignancy (17%). In 19% of patients, however, no discernable cause of FUO was found.
With regard to the patient we describe, many infectious etiologies had already been excluded

by the negative results of blood and urine cultures, normal transaminase values, and the absence of occult abscesses revealed by computed tomographic scans of the chest, abdomen, and pelvis. Endocarditis was unlikely because the patient had 5 sets of negative blood cultures and a normal transthoracic echocardiogram. Therefore the FUO in this patient satisfied only 1 minor Duke criterion for endocarditis, fever greater than 38.0°C. Evaluation for glomerulonephritis was important in this patient because of the possibility of septic emboli or immune complex damage from endocarditis.

Additional History

In addition to subacute bacterial endocarditis, vasculitis was considered by the general medicine team to be a possible etiology for the patient's renal failure and prolonged intermittent fevers. Results of her initial laboratory studies included antinuclear antibodies 1:80, C3 141 mg/dL (normal 90-180 mg/dL) and C4 18 mg/dL (normal 16-47 mg/dL). Results of urinalysis and sediment demonstrated 2+ protein, 30-50 red blood cells (RBC), 17 white blood cells, dysmorphic RBC, and many granular casts. Her fractional excretion of sodium was 1.2%. Inflammatory markers were elevated, including an erythrocyte sedimentation rate of 125 mm/h, C-reactive protein > 150 mg/dL, and ferritin 1077 ng/mL.

Differential Diagnosis

The patient was initially treated for possible AIN, but her creatinine continued to be elevated despite treatment with prednisone. Urinalysis demonstrating 2+ protein and hematuria as well as dysmorphic RBC in the urine sediment suggested the presence of glomerulonephropathies. Broadly, glomerulonephritis is defined by intraglomerular inflammation with cellular proliferation and infiltration by peripheral leukocytes, which results in hematuria.[13] The hematuria includes RBC, which are frequently dysmorphic, and usually includes RBC casts. The 5 clinical syndromes related to glomerulonephritis are asymptomatic hematuria, acute glomerulonephritis, rapidly progressing glomerulonephritis (RPGN), nephrotic syndrome, and chronic glomerulonephritis. Glomerulonephritis can occur as a primary renal disease or may be related to a systemic process such as vasculitis.[14]

In this patient, given the 5-week course of fevers and renal insufficiency punctuated by a more acute increase of creatinine during a 1-week period, her presentation was most concerning for RPGN. RPGN is a clinical syndrome that includes evidence of glomerulonephritis with a 50% reduction in glomerular filtration rate or a doubling of creatinine within 2 months. On the basis of renal biopsy results RPGN can be diagnosed as either crescentic or noncrescentic. A diagnosis of crescentic glomerulonephritis requires a renal biopsy sample demonstrating extensive (>50%) crescents. RPGN can occur in the setting of other glomerulonephritides and systemic diseases, but it is helpful to consider 3 broad categories for the pathologies associated with RPGN (Table 1).[15] Type 1 is anti–glomerular basement membrane (anti-GBM) disease. Type 2, immune-complex RPGN, is often associated with an underlying disease such as endocarditis, IgA nephropathy, postinfectious glomerulonephritis, lupus nephritis, or mixed cyroglobulinemia. Type 3 is pauciimmune, in which no immune deposits are revealed by electron microscopy or immunofluorescence. The pauciimmune RPGN represent a group of vasculidities, either systemic or limited to the kidney, of which many are positive for antineutrophil cytoplasmic antibody (ANCA). Included in the type 3 category is the spectrum of conditions associated with small-to-medium–vessel ANCA-positive vasculitis, including Wegener granulomatosis and

Table 1: Classification of Rapidly-Progressing Glomerulonephritis (RPGN)

Type of RPGN	Etiologies
Type 1: Anti–glomerular basement membrane	Seen with antibodies to glomerular basement membrane.
Type 2: Immune complex	Occurs with immune complex formation due to underlying diseases. Immune complex materials are deposited in the glomerulus.
Type 3: Pauciimmune	Related to antineutrophil antibodies in the setting of vasculitis.

microscopic polyangiitis.[16] When no apparent secondary cause of RPGN is identified, the RPGN is considered idiopathic. Three types of idiopathic RPGN have been identified based on pathologic and serologic features. Differentiating the underlying pathologies associated with the syndrome of RPGN is done by integrating clinical history and results of laboratory analysis (such as serological tests for anti-GBM and ANCA)

The use of complement levels can assist in differentiating etiologies of glomerulonephritis. A low complement level is seen in patients with immune complex glomerulonephritides, whereas patients with ANCA-positive and anti-GBM RPGN have normal complement levels.

In this patient an urgent renal biopsy was required to determine if glomerulonephritis was the source of her renal failure. Additional serological tests for ANCA and anti-GBM were performed.

Diagnostic Procedure and Diagnosis

The patient underwent renal biopsy, and tissue samples were examined with light, immunofluorescence, and electron microscopy. On light microscopy, 82% of nonsclerotic glomeruli demonstrated crescent formation, with fibrocellular crescents predominating over cellular ones. Glomerular tufts demonstrated widespread fibrinoid necrosis. There was a marked interstitial mixed inflammatory infiltrate, moderate tubular atrophy, and moderate-to-severe interstitial fibrosis. A single interlobular artery demonstrated mural fibrinoid necrosis (Figure 1).

The material submitted for immunofluorescence microscopy contained a single nonsclerotic glomerulus. There was 1+ linear GBM staining with antiserum specific for IgG. Ultrastructural analysis was negative for immune-complex–type deposits.
An enzyme-linked immunosorbent assay (ELISA) for anti-GBM was strongly positive at 170 units (normal range 0-20 units). Indirect immunofluoresence for ANCA was also positive at a titer of 1:80 in a perinuclear pattern. Because of the unusual features in this case (very high anti-GBM level with only 1+ GBM staining on immunofluorescence; biopsy sample with concurrent ANCA positivity and arteritis), serum samples were sent to a reference laboratory for Western blot analysis to confirm the specificity of the anti-GBM antibody. The patient serum reacted with monomeric and dimeric forms of the Goodpasture antigen (NC1 domain of the alpha 3 chain of type IV collagen, Col4A3). The findings in this case were diagnostic for necrotizing crescentic glomerulonephritis associated with both anti-GBM and ANCA.

DISCUSSION

The light microscopic findings in this case revealed a morphologic pattern diagnostic for crescentic glomerulonephritis, which is seen with most RPGN. Information from light, immunofluoresence, and electron microscopy as well as serologic analyses allows for differentiation of pauciimmune glomerulonephritis from anti-GBM disease.

The glomerular changes associated with pauciimmune glomerulonephritis and anti-GBM disease are indistinguishable when samples are viewed with light microscopy. Both diseases are characterized by variable numbers of crescents. Extraglomerular vasculitis is not seen in pure anti-GBM disease but may be seen in so-called double-positive disease (see below) and pauciimmune and lupus glomerulonephritis.

Immunofluorescence microscopy may be the most useful modality in differentiating these diagnostic entities. Pauciimmune glomerulonephritis is defined by its paucity or lack of staining for glomerular immunoglobulins. Anti-GBM disease is characterized by intense linear staining (3+-4+ on a scale of 0-4+) of the GBM with antiimmunoglobulins. In the vast majority of cases, IgG is the dominant immunoglobulin, although rare cases of IgA-dominant anti-GBM disease have been described.[17] Linear staining of tubular basement membranes may be observed as well. The continuous staining is attributable to the fact that the implicated antibody has specificity for an antigen within these basement membranes. The intensity of the immunostaining is important because less intense linear staining may be nonspecific and is seen in certain disease states, including diabetic nephropathy.[18]

Electron microscopy findings are nonspecific in pauciimmune glomerulonephritis and anti-GBM disease. In most cases, electron microscopy reveals a lack of immune complex deposits, which correlates with the findings on light and immunofluorescence microscopy. Given an otherwise compelling clinicopathologic picture, the presence of electron dense deposits does not obviate a diagnosis of

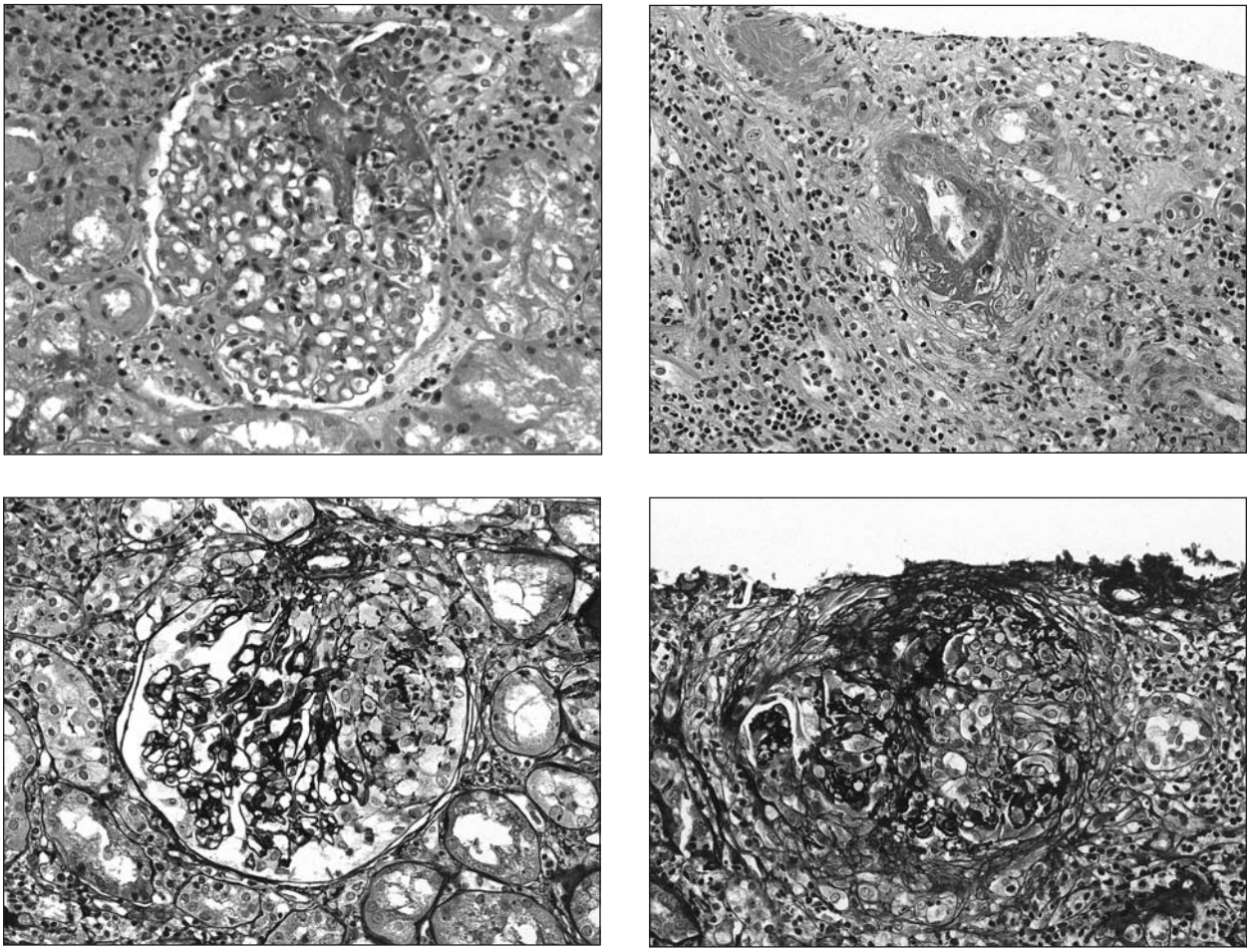

Figure 1. Necrotizing crescentic glomerulonephritis. Top left, This glomerulus demonstrates segmental fibrinoid necrosis of capillary loops (in the upper right-hand aspect). The remainder of the glomerulus is unperturbed (hematoxylin and eosin, original magnification ´ 200). Top right, Mural fibrinoid necrosis identifiable in an interlobular artery. This is not a feature of pure anti–glomerular basement membrane disease but may be seen in so-called double-positive disease (hematoxylin and eosin, original magnification ´ 200). Bottom, left to right, Crescent involvement was visible with the majority of nonsclerotic glomeruli. Crescents ranged from early lesions partially involving the glomerulus and associated with relative preservation of glomerular architecture (left) to those that obliterated the glomerulus (right) (Jones silver, original magnification ´ 200).

pauciimmune glomerulonephritis or anti-GBM disease, because these diseases may coexist with immune-complex disease (eg, coexistent anti-GBM disease and membranous glomerulonephritis).[19]

In this case serological test results for both ANCA and anti-GBM were positive. The most sensitive and specific serological assay for anti-GBM disease is the direct ELISA for anti-GBM antibodies in the serum. The sensitivity of this assay is 95%-100% and the specificity is 90%-100%, depending on the commercial brand used. Confirmatory Western blot testing can be done to verify the specificity of the ELISA assay.[20] In the setting of crescentic glomerulonephritis, the presence of anti-GBM is virtually diagnostic for anti-GBM glomerulonephritis. The existence of double-positive disease (ie, positive for both anti-GBM and ANCA, as in this case) has been increasingly recognized, and thus in the setting of RPGN, testing for both is recommended. Although ANCA is positive in approximately 30% of patients with anti-GBM, only 5% of patients with an ANCA will have concurrent anti-GBM.[21]

Discussion of Anti-GBM Disease

Historically, pulmonary hemorrhage and glomerulonephritis were described in young men following influenza infection, as first identified by Goodpasture in 1918.[22] It is likely that some

of these cases were anti-GBM disease; however, confirmatory laboratory tests were not available at that time. More recently, in 1958, Stanton and Tang reported a series of young men with pulmonary hemorrhage and glomerulonephritis, similar to Goodpasture's original description. The pathogenesis of the syndrome was not well understood, however, until the discovery of anti-GBM antibodies in 1967. Anti-GBM is a rare disease, with an incidence of 1 case per 1 million patient years.[23] The disease affects the capillaries in specific organs containing the autoantigen, the alpha-3 chain of type IV collagen.[24]The hallmark of this disease is renal involvement, frequently with crescents and RPGN and/or pulmonary hemorrhage. Patients with both pulmonary and renal involvement have Goodpasture syndrome. It has been postulated that patients with pulmonary involvement have underlying lung injury that allows the anti-GBM antibodies to reach the alveolar basement membrane.[25]

The early diagnosis of anti-GBM disease is difficult when there is only renal involvement. Interestingly, in patients with anti-GBM disease, 30%-40% have isolated renal involvement. However, anti-GBM disease has been estimated to cause up to 20% of cases with RPGN.[12] Isolated renal anti-GBM disease usually occurs in female patients approximately 60 years old and involves renal failure with hematuria and glomerulonephritis. Usually the presence of more systemic symptoms including fever indicates concurrent systemic ANCA-positive vasculitis.[2]

Patient Follow-up

With the results of the renal biopsy demonstrating crescentic glomerulonephritis and a clinical diagnosis of RPGN, the patient was initially started on intravenous methylprednisolone for 3 days then transitioned to prednisone 60 mg daily, cyclophosphamide 75 mg daily, and plasmapheresis. Ultimately the patient required intermittent hemodialysis after the third course of plasmapheresis because her renal function did not improve. After 1 week of plasmapheresis serological analysis results showed that the anti-GBM antibody had significantly decreased from 170 units to 62 units.

The patient's diagnosis of anti-GBM disease was complicated by renal biopsy results that showed only 1+ IgG deposits. Results of both ELISA and Western blot analyses, however, confirmed peripheral blood positivity for anti-GBM. It remains a possibility that the patient had positive anti-GBM due to cross reactivity with the Col4A1/2 and therefore did not have antibody response to the Col4A3 found in anti-GBM disease. If that is the case, then her diagnosis would be ANCA-positive vasculitis. Because the ANCA titer peaks and falls, the interpretation of the titer depends on timing, and this patient's ANCA level was not retested after treatment for her glomerulonephritis.

After discharge from the hospital, the patient was maintained on intermittent hemodialysis and cyclophosphamide for treatment of crescentic glomerulonephritis. Her prednisone was tapered off after she completed 3 weeks of daily prednisone at 60 mg.

Discussion of Management of RPGN due to Anti-GBM disease

Prior to the use of plasmapheresis, RPGN due to anti-GBM disease conferred a poor prognosis.[27] Only 1 randomized controlled clinical trial compared immunosuppressive bone plasmapheresis plus immunosuppression and anti-GBM disease. This trial showed no difference in outcomes of renal disease, and results indicated that both treatment modalities were equally effective for the pulmonary hemorrhage, in addition to standard immunosuppressive therapy, with faster decline in anti-GBM antibody titers, lower serum creatinine after therapy, and fewer patients progressing to renal failure.[28] The outcomes of patients who present with RPGN due to anti-GBM disease is critically dependent on their creatinine concentration at the time of presentation and percentage of crescents.[22,29] For patients who presented with a creatinine less than 5.7 mg/dL and were initially treated with glucocorticoids, cyclophosphamide, and plasma exchange, the 1-year survival was 100% and the 1-year renal survival was 95%.[30] Patients who required dialysis at the time of diagnosis had a significantly worse 1-year survival as well as a lower rate of renal function recovery.

The optimum duration of treatment is debated owing to variability in the resolution of the autoantibody. Some experts advocate monitoring serial anti-GBM antibody levels until they are negative on 2 successive evaluations and then tapering the immunosuppressants at that time. If the anti-GBM antibody levels remain elevated, the course of therapy with cyclophosphamide is continued for 4-6 months.

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Wide Complex Rhythm in a Hospitalized Orthopedic Patient

William J. Brady, MD, Professor of Emergency Medicine and Medicine

In patients with hyperkalemia, disruption of cardiac electrical activity by increased serum potassium can lead to electrocardiographic (ECG) abnormalities and dysrhythmias. These abnormalities are dependent on the magnitude and rapidity of the increase of potassium, however, and with mild to moderate increases in serum potassium the ECG may appear normal. Management of hyperkalemia is based on the patient's clinical situation, including ECG findings. Clinicians must be familiar with ECG patterns associated with hyperkalemia because of their importance in guiding both the urgency and the magnitude of treatment interventions.

CASE REPORT

The patient was a 47-year-old man with a history of hypertension, alcohol abuse, and mild renal insufficiency; home medications included hydrochlorothiazide and aspirin. Four days prior to the events presented in this report the patient had been admitted to the orthopedic surgery service from the emergency department with a right femur fracture sustained in a ground-level fall likely related to alcohol intoxication. At that time, the patient was clinically intoxicated with ethanol and he demonstrated findings consistent with the extremity fracture. Laboratory study results were normal with the exception of a serum creatinine concentration of 2.1 mg/dL (approximately the patient's baseline creatinine value). Creatinine phosphokinase and serum electrolyte concentrations were normal. The patient was admitted for orthopedic surgery and underwent operative fixation of the fracture later that day. His postoperative course was complicated by mild alcohol withdrawal, which was managed with intravenous and oral benzodiazepine. The patient's postoperative pain was managed with intravenous morphine and ketoralac. On postoperative day 2 the patient was recovering well and performing physical therapy; laboratory study results were significant for a serum creatinine of 2.5 mg/dL.

On the evening of postoperative day 3, the patient was noted to be lethargic; his vital signs were significant for a blood pressure of 140/90 mm Hg and a pulse rate of 65 beats/min, respirations of 20/minute, temperature of 36.8°C, and oxygen saturation of 95% on room air. Benzodiazepine therapy was discontinued while the patient was observed. On the morning of hospital day 4, his mental status had worsened.

The hospitalist was consulted regarding the patient's mental status alteration and slow pulse. On arrival at the patient's bedside, the hospitalist found that the patient was lethargic but would arouse in response to verbal and physical stimuli. Vital signs were significant for a pulse of 50 beats/min; findings from the remainder of the examination, other than the mental status, were consistent with the patient's postoperative situation. Results of a bedside glucose measurement were normal. The patient did not respond to treatment with 0.4 mg of intravenous naloxone, and ECG, chest radiograph, and laboratory studies were ordered.

ECG Findings

The ECG rhythm strip (Figure 1) demonstrated a wide, regular QRS complex rhythm with a ventricular response of approximately 50 beats/min. A 12-lead ECG (Figure 2) demonstrated similar findings.

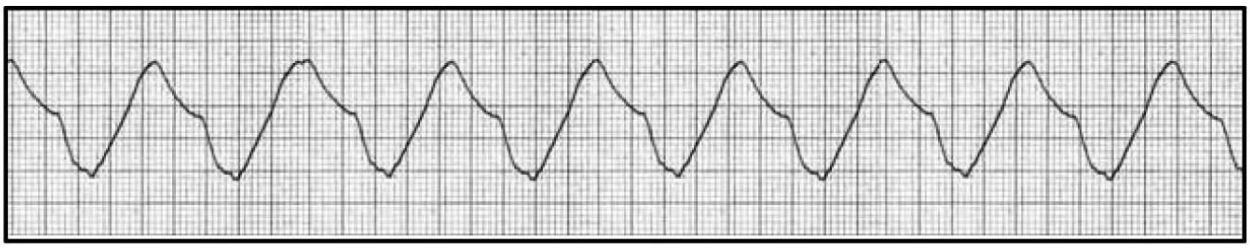

Figure 1. Patient electrocardiograph rhythm strip on presentation shows bradycardia with a wide complex QRS rhythm.

On review of the ECG findings (Figures 1 and 2), the hospitalist noted the rhythm and immediately was concerned about the possibility of severe hyperkalemia. As such, initial therapy aimed at correction of the cardiac rhythm was initiated. The rhythm strip in Figure 3 was noted approximately 8 minutes after initiation of therapy.

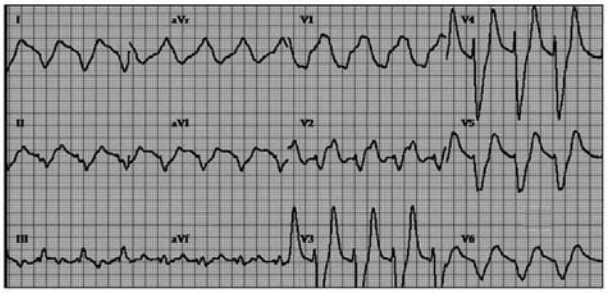

Figure 2. 12-Lead electrocardiograph shows a wide QRS complex rhythm.

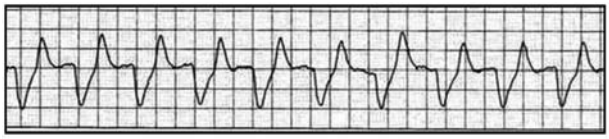

Figure 3. 12-Lead electrocardiograph rhythm strip after approximately 8 minutes of focused therapy. Note that the QRS complex has narrowed but is still quite wide; P waves are still not apparent.

DISCUSSION

The ECG manifestations associated with hyperkalemia are numerous and involve both morphologic change and dysrhythmia.[1,2] In a very basic sense, elevated serum potassium values lead to a disruption of cardiac electrical activity—both impulse formation and conduction. Thus the ECG findings in hyperkalemic patients are a manifestation of these altered pacemaker and conduction system functions. These morphologic alterations include prominent T waves, PR-interval prolongation, loss of the P wave, QRS-complex widening, and the sinusoidal QRS complex configuration. The rhythms include both bradycardia and tachycardia. Bradycardia can present both with and without intra- and interventricular conduction block. Tachycardia most frequently involves ventricular dysrhythmia. An ominous dysrhythmia that is strongly suggestive of severe hyperkalemia is the sinoventricular rhythm; it is characterized by loss of the P wave and sine-wave QRS complex, most often with a slow ventricular response.

The ECG abnormalities associated with hyperkalemia are dependent on both the magnitude of the potassium elevation and the rapidly of its development. Increasingly higher levels of serum potassium are associated with a greater potential for ECG abnormality and dysrhythmia. Furthermore, a relatively slower development of hyperkalemia tends to produce less ECG manifestation, whereas sudden increases in the potassium concentration are likely to cause more significant abnormality in the ECG.[1,2] Of course, the ECG may not demonstrate these classic abnormalities in all instances. In fact, the ECG may appear either normal or nonspecifically abnormal in patients with mild to moderate serum potassium elevations. The ECG may also demonstrate abnormalities that are not necessarily considered classic manifestations of hyperkalemia, such as a complete heart block and/or bundle branch block.[3,4]

The prominent T wave (Figure 4) is considered the first significant ECG manifestation of hyperkalemia.[1,2] Minimal elevations in the serum potassium enhance or accentuate repolarization of the myocyte, which is demonstrated on the ECG by alterations in the T wave (Figure 4), most often producing the prominent T wave.[1,2] The T wave is described as tall and narrow with a symmetric structure (Figure 4). The polarity of the T wave may also change, particularly in patients with T-wave inversions related to left ventricular hypertrophy, which show a pattern known as the "strain pattern." These inverted T waves will become upright, causing a "pseudo-normalization" pattern.[1,2]

As noted, continued elevations in the serum potassium will affect cardiac impulse formation and electrical conduction; in essence, hyperkalemia produces a slowing or prolongation of cardiac conduction. All cardiac myocytes are sensitive to elevated potassium levels. Atrial tissue, however, is significantly more sensitive to the effects of hyperkalemia. PR-interval prolongation occurs and is followed by a dampening of the P wave. At progressively higher serum potassium levels, the QRS complex widens (Figures 1-3 and 5), at times resembling a bundle branch block. Eventually, the QRS complex blends with the T wave, forming a "sine-wave" or sinusoidal, structure on the ECG (Figures 1 and 2). At this point, the P wave further lessens in amplitude, ultimately disappearing with continued serum potassium elevation (Figures 1 and 2). Despite the disappearence of the P wave on ECG, sinus rhythm continues with maintained sinus node activity; at this point, the sine wave with loss

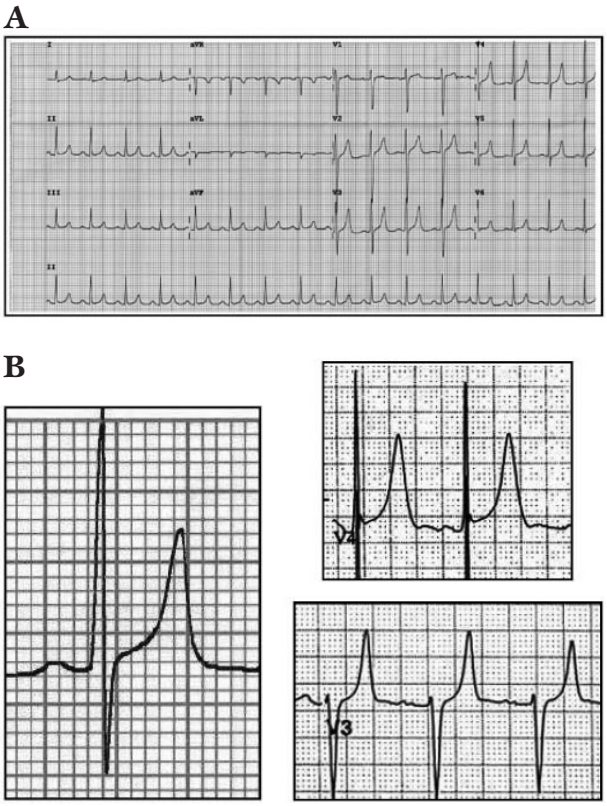

Figure 4. A, 12-Lead electrocardiograph (ECG) obtained 30 minutes after initiation of therapy in the case patient. Prominent T waves in leads V3 to V4 are frequently seen in hyperkalemia. B, Prominent T-wave configurations noted in hyperkalemia. Note the tall, narrow, and symmetric structure of the T wave, a classic ECG finding in patients with hyperkalemia.

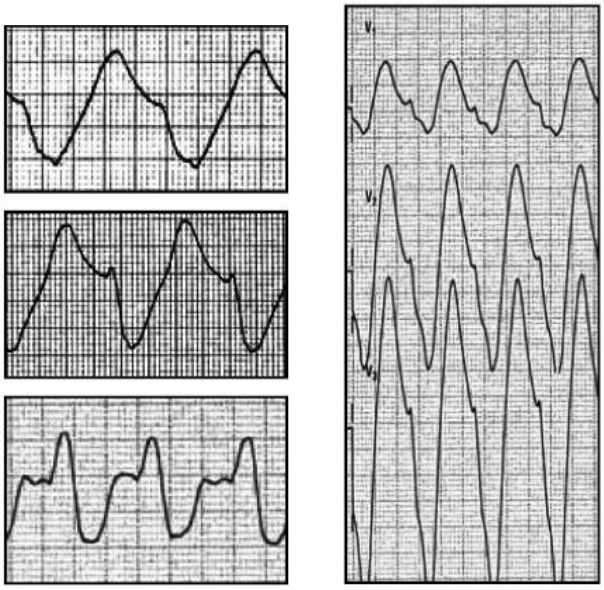

Figure 5. Widened QRS complexes resulting from hyperkalemia.

of the P wave characterizes the "sinoventricular" rhythm of hyperkalemia (Figures 1, 2, and 5).[1,2] Progressive increases in the potassium levels eventually result in ventricular fibrillation and asystole.[1,2]

The management of hyperkalemia (Table 1 and the algorithm in Figure 6) is guided in large part by the patient's clinical situation, including ECG findings. In fact, the ECG should guide both the urgency and the magnitude of the interventions. The management of hyperkalemia consists of 3 primary goals: stabilization of the myocardial cell membrane, shift of the potassium from the vascular to intracellular space, and removal of the potassium from the body. Response to therapy is often prompt and is demonstrated by visual changes on the ECG monitor (Figure 7).

Management issues for hyperkalemia are presented in Table 1, and the algorithm in Figure 6 shows a suggested order of therapy priority.[5]

CONCLUSION

The hospitalist reviewed the patient's ECG (Figures 1 and 2), noted the rhythm, and was immediately concerned about the presence of severe hyperkalemia. The 12-lead ECG (Figure 2) demonstrated the absence of P waves and markedly widened QRS complexes with a sine wave morphology. Note that the T wave had fused with the widened QRS complex to form the sine wave. This rhythm is consistent with the sinoventricular rhythm of severe hyperkalemia.

On the basis of these ECG findings, the hospitalist treated the patient with intravenous medications that included calcium chloride, regular insulin, dextrose, and sodium bicarbonate; the patient also received nebulized albuterol and oral polystyrene-binding resin; arrangements for emergent hemodialysis were also made. Laboratory studies were significant for a serum potassium of 8.1 mmol/L. Continuous rhythm monitoring demonstrated some degree of QRS-complex narrowing (Figure 3).

While the patient awaited hemodialysis, a repeat ECG was performed and demonstrated a marked narrowing of the QRS complex with the development of very prominent T waves in the anterolateral leads (Figure 4A). During this period, the patient also received additional therapy—insulin, 50% dextrose, sodium bicarbonate, and nebulized albuterol.

The patient underwent hemodialysis with no problems. A repeat ECG performed 24 hours later demonstrated a normalization of T-wave abnormalities. The sequential ECG abnormalities seen in this patient as therapy was administered are shown in Figure 7, and study questions for review are presented in Table 2.

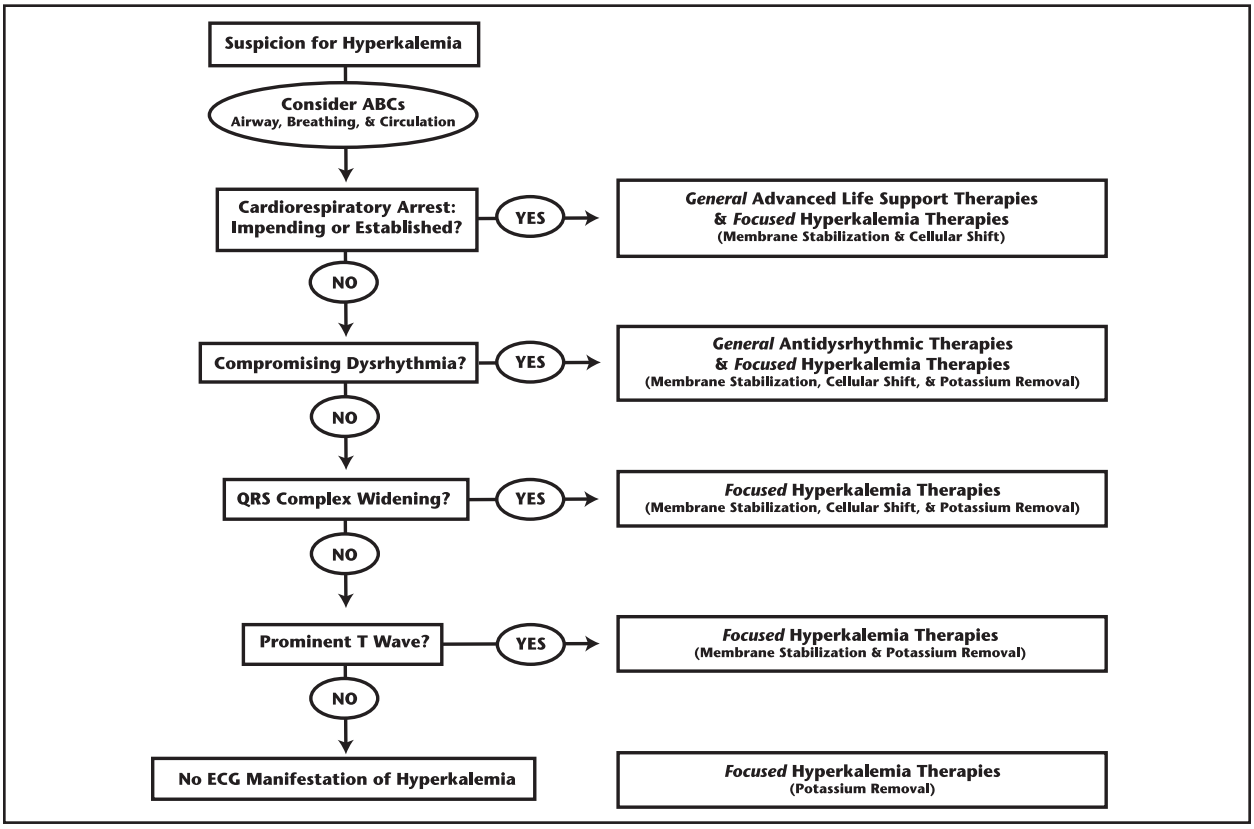

Figure 6. Algorithm showing a suggested therapeutic approach to patients with hyperkalemia. Note that the electrocardiographic (ECG) findings play a central role in this treatment algorithm.

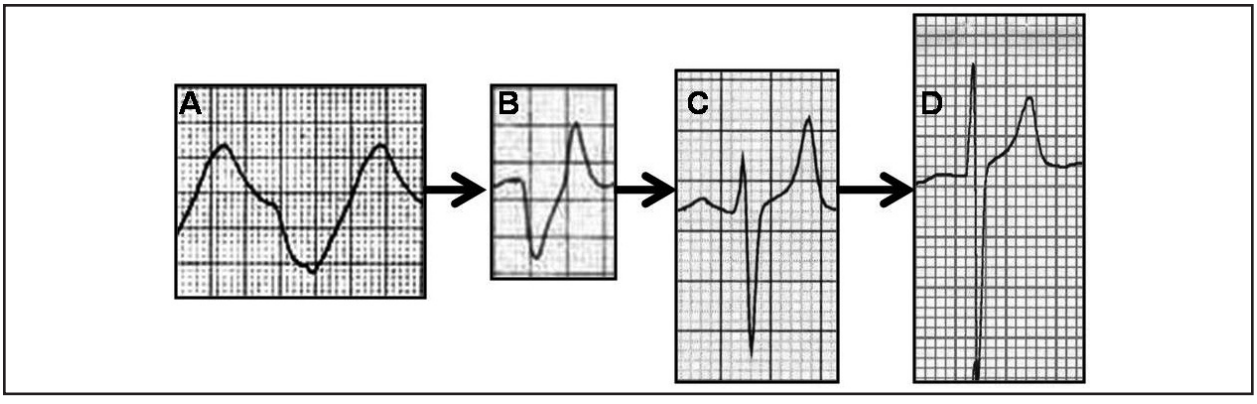

Figure 7. Progressive electrocardiographic (ECG) changes demonstrate improvement in the hyperkalemic patient in response to therapy. A, At time 0 the sine-wave configuration appeared without an obvious P wave (the sinoventricular rhythm). B, At 8 minutes after treatment was administered the QRS complex was still visibly wide, but narrowing had occurred during a very brief time period after initiation of therapy. The P wave was still not apparent. C, At 30 minutes the QRS had markedly narrowed and the P wave had appeared. The T wave was quite prominent now and was the only manifestation of hyperkalemia on this ECG. D, At 6 hours, after the patient underwent complete hemodialysis, this normal ECG was obtained.

Table 1. Therapeutic Agents and Interventions in the Case Patient with Suspected Hyperkalemia.*

Therapeutic Goal	Agent	Dose and Administration	Comments
Membrane stabilization	Calcium chloride (13.6 mEq/10 mL) Calcium gluconate (4.6 mEq /10 mL)	10 mL IV over 1 minute in nonarrest. IV push in cardiac arrest, maximum dose 20 mL in 30 minutes	Should be initial medication used in treatment strategy. Caution in hyperkalemia-related digoxin toxicity.
Intracellular shift	Dextrose/insulin	Dextrose 50 grams IV push; insulin (regular) 10 units IV push	Mechanism: stimulation of the cellular glucose pump. Will lower serum level by approximately 1 mmol/L over 20 to 60 minutes, with greatest reduction occurring in first 20 minutes. Effect is transient with repeat administration potentially necessary.
	Sodium bicarbonate	1 mEq/kg body weight IV. IV over 20-minute period in nonemergent setting; IV push in cardiac arrest or significant dysrhythmia	Mechanism: hydrogen-potassium exchange. Reduction is dependent on pH change: for every 0.1 increase in serum pH, the serum potassium should fall by 0.5 mmol/L. Effect is transient.
	Magnesium sulfate	1 to 2 grams IV over 5 to 20 minutes	Mechanism: cAMP-mediated sodium-potassium pump. Rapid reduction as early as 5 minutes with 0.5 mmol/L reduction. Effect is transient.
	Albuterol	Standard metered "respiratory dose" via nebulizer	Mechanism: cAMP-mediated sodium-potassium pump. Reduction by 0.5 to 1.0 mmol/L over 30 minutes; after 30 minutes, effect wanes rapidly. Other beta-agonists have similar impact.
Potassium removal	Sodium polystyrene	30 to 60 g orally or per rectum	Impact is modest at best with relatively slow onset of action.
	Normal saline	Determined by clinical situation	Consider volume status and ability to make urine. Can also produce intracellular shift.
	Furosemide	20 mg IV for furosemide-naïve patients; prescribed daily dose IV for patients using furosemide	Impact is modest at best with relatively slow onset to action.
	Hemodialysis	—	Necessary therapy for patients with abnormal QRS complex or significant dysrhythmia.

*Therapy should be initiated empirically based on the appearance of the ECG. As therapy is being administered, laboratory confirmation of the metabolic disorder should be obtained. IV indicates intravenous.

Table 2. Electrocardiographic (ECG) Findings in the Case Patient: Study Questions

1. Considering the patient's situation and his ECG rhythm strip (Figure 1), what is the most likely rhythm diagnosis in this patient?

   A. Junctional bradycardia
   B. Ventricular paced rhythm
   C. Idioventricular rhythm
   D. Sinoventricular rhythm

Correct answer: D. Sinoventricular rhythm

The other rhythm possibilities offered are incorrect for the following reasons:

A. Junctional bradycardia: In a junctional rhythm, the QRS complex is most often narrow.
B. Ventricular paced rhythm: In a paced rhythm, evidence of pacer spikes is most often seen; furthermore, the QRS complex in Figure 1 is too wide for a typical ventricular pace rhythm.
C. Idioventricular rhythm: The rhythm in Figure 1 could represent an idioventricular rhythm; nevertheless this answer is unlikely in that the QRS complex is too wide and the rate is greater than anticipated in an idioventricular rhythm.

2. What is the most likely etiology of this patient's cardiac rhythm?

   A. Hyperkalemia
   B. Acute myocardial infarction
   C. Acute central nervous system hemorrhage
   D. Systemic fat embolism
   E. Sodium channel blocker toxicity

Correct answer: A. Hyperkalemia

Answers B. Acute myocardial infarction, C. Acute central nervous system hemorrhage, and D. Systemic fat embolism are incorrect because these conditions are all unlikely causes of such a rhythm disturbance. Answer E. Sodium channel blocker toxicity is incorrect because although this condition can produce rhythm disorders with widened QRS complexes, this QRS complex configuration is not consistent with such a rhythm, as noted in the explanation given for the previous question.

3. What combination of therapies and order of the therapies is most appropriate for this patient at this point in the evaluation (all agents are administered intravenously unless otherwise stated)?

   A. Transcutaneous pacing, calcium, atropine, epinephrine, dextrose, tenecteplase
   B. Calcium, dextrose, insulin, sodium bicarbonate, nebulized albuterol, magnesium sulfate
   C. Magnesium sulfate, calcium, dexamethasone, furosemide, normal saline, morphine sulfate
   D. Normal saline, dopamine, atropine, transcutaneous pacing, calcium, tenecteplase

Correct answer: B. Calcium, dextrose, insulin, sodium bicarbonate, nebulized albuterol, magnesium sulfate. The administration of the agents in this listed order is most appropriate. Calcium stabilizes the myocyte, reducing the potential for ventricular dysrhythmia,; and dextrose, insulin, sodium bicarbonate, nebulized albuterol, and magnesium sulfate all promote an internal shift of the serum potassium into the cell. Refer to Table 1 and the algorithm in Figure 6 for a focused discussion on the most appropriate approach to hyperkalemia.

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Increased Incidence of Clostridium difficile*–Associated Diarrhea and Fluoroquinolone Use at the University of Virginia Health System*

Laurie Archbald-Pannone, MD, MPH, Assistant Professor of Medicine, Division of General Medicine, Geriatrics, and Palliative Care
Cirle Alcantara-Warren, MD, Assistant Professor of Medicine, Division of Infectious Disease and International Health
Jason Lyman, MD, Assistant Professor of Public Health Sciences, Division of Informatics
Richard L. Guerrant, MD, Professor of Internal Medicine, Division of Infectious Diseases and International Health

ABSTRACT

Objective: *Clostridium difficile* epidemics and increased use of fluoroquinolones have highlighted the importance of determining the rate of fluoroquinolone-associated *C. difficile* infection (CDI).

Methods: We compared the use of multiple antibiotics among local inpatients and the association of each with a diarrheal stool specimen, intestinal inflammation (as indicated by a positive test for lactoferrin), and positive *C. difficile* toxin assay results.

Results: From August 2002 to December 2005, there was a 39% increase in stool specimens tested for *C. difficile* ($P \leq 0.001$) as a marker of antibiotic-associated diarrhea, and a 111% increase in CDI ($P \leq 0.01$). In 2004, ciprofloxacin was used more than moxifloxacin, ceftriaxone, or clindamycin and more diarrheal stool specimens were tested for *C. difficile* from patients treated with ciprofloxacin than from patients treated with other antibiotics ($P \leq 0.001$). The rate of *C. difficile* testing as a marker of antibiotic-associated diarrhea was 50% more with ciprofloxacin than with ceftriaxone and more than 2 times that with clindamycin treatment. The rate of CDI in patients treated with ciprofloxacin was 1.5 times that of patients treated with ceftriaxone and 3.8 times that of patients treated with clindamycin. Of all CDI patients tested for lactoferrin, nearly half had fecal specimens that tested positive, suggesting no reduction in disease severity.

Conclusion: At our institution, CDI has increased steadily, more than doubling since 2002, and is associated with an increase in ciprofloxacin use.

INTRODUCTION

Clostridium difficile is a gram positive, anaerobic bacterium that is a major cause of nosocomial diarrhea. *C. difficile* infection (CDI) accounts for 10%-20% of cases of antibiotic-associated diarrhea and nearly all cases of antibiotic-associated pseudomembranous colitis.[1] *C. difficile* causes a toxin-mediated enteric disease via toxins A and/or B.[2] Established risk factors for CDI include age, hospitalization, and recent use of antibiotics. The incidence of *C. difficile* has been increasing during the past few decades. In the 1980s, the Centers for Disease Control and Prevention (CDC) reported that *C. difficile* was identified in the stool of 7% of hospitalized patients and that 21% of these patients had acquired this infection while hospitalized. Nearly 20 years later, the CDC reported that the incidence of CDI in the United States had been steadily increasing from 1987 to 2001.[1] In 1998, Johnson and Gerding reported that *C. difficile* colonies were found in few patients at the time of hospital admission but were found in nearly half of patients 4 weeks later. In 2000, an outbreak of severe CDI that occurred in a Pittsburgh hospital resulted in an increase in cases from 2.7 to 6.8 cases per 1000 patients discharged from the hospital, as well as an increase in disease severity reflected by an increase in the number of colectomies and deaths during the 2-year period after the outbreak.[4] This outbreak uncovered the emergence of a fluoroquinolone-resistant strain of *C. difficile* with genetic mutations found to be associated with toxinotype III, NAP1/027/BI, including binary toxin (*cdtA* and *cdtB*) and a deletion in the *tcdC*-negative regulator (BI strain). In 2002, another outbreak occurred in Québec. For 2 years, hospitals in Québec saw an increase of 5 times the national average in the number of

patients admitted for CDI and 3 times the 30-day mortality rate. This epidemic was found to be associated with the same BI strain.[4]

Although virtually any antibiotic can be implicated as a cause of CDI, clindamycin and broad-spectrum cephalosporins have most commonly been cited as leading causes for CDI. Now that fluoroquinolones are the most commonly prescribed antibiotic in the United States, the need to define the rate of fluoroquinolone-associated CDI is greater than ever.[4] Ciprofloxacin, one of the most commonly prescribed fluoroquinolones, is used to treat a variety of microbial infections. Recent reports of hypervirulent strains of *C. difficile* in the Pittsburgh and Québec epidemics have linked these worrisome strains to fluoroquinolone-resistant *C. difficile*.[4-7] In 2003, McCusker et al used a multivariable regression model to show that treatment with fluoroquinolones was the strongest risk factor for CDI (odds ratio 12.7; 95% confidence interval 2.6-61.6).[4-7] Therefore, we examined major antimicrobial use and its association with CDI at our institution, the University of Virginia Health System (UVA) from 2002 to 2005.

MATERIALS AND METHODS

In this study we examined the use of ciprofloxacin among other antimicrobial agents at our institution and the association of each with diarrhea (defined as unformed [diarrheal] stool submitted for laboratory testing for *C. difficile*), inflammation (defined as positive fecal lactoferrin detected by use of the IBD-CHEK enzyme-linked immunoassay (TechLab, Blacksburg, VA), and documented positive *C. difficile* toxin A or B detected via enzyme-linked immunoassay (ProSpecT; Remel, Lenexa, KS). We evaluated the rate of ciprofloxacin-associated CDI at UVA by use of the UVA clinical data repository (CDR). University of Virginia Institutional Review Board approval for use of the CDR was obtained for this study, which was categorized as research not involving human subjects (protocol #1651).

Information systems like the CDR, an institutional enterprise-wide data warehouse, have traditionally been used for financial analysis and strategic marketing in medical centers, but are increasingly being used as sources of data for clinical investigation and health services research. Like most academic data warehouses, the UVA CDR includes data derived from multiple clinical, administrative, and financial systems within our institution and supplemented by external data sets such as vital registry data from the state health department. A less common feature of the UVA CDR is a powerful web-based user interface that allows users to independently conduct flexible ad hoc queries or work collaboratively with data analysts.[8]

We examined deidentified microbiological data in the CDR obtained from August 2002 to December 2005 from all sequential patients treated at UVA who had documented positive *C. difficile* toxin A or B. A sample submitted for *C. difficile* testing was defined as a diarrheal stool specimen analyzed to identify possible antibiotic-associated diarrhea. A positive fecal lactoferrin assay was used as a marker of intestinal inflammation.[9] We further analyzed data on all patients treated with ciprofloxacin, the most commonly used fluoroquinolone on the hospital formulary; ceftriaxone (a third-generation cephalosporin); and clindamycin, in 2004. The database query included any patient who had received at least 1 dose of antibiotic and did not account for length of treatment or treatment with multiple antibiotics. The χ^2 test was used to determine significance, set at a level of $P < .05$.

RESULTS

With the exception of 2002, when the laboratory assays for *C. difficile* and lactoferrin were initiated at the main hospital clinical microbiology laboratory for the latter part of the year, the populations for each year were comparable. Details are listed in Table 1. There was a statistically significant ($P < .001$) increase of 30% in number of patients with a diarrheic sample submitted for microbiological

Table 1. Number of Patients Evaluated for Clostridium difficile Infection at the University of Virginia Health System

Year	Inpatients	Diarrheal Specimen	Diarrheal Specimen with C. difficile Toxin +	C. difficile + for All Inpatients	Lactoferrin + Among All Tested Patients
Aug-Dec 2002	10,760	426 (3.96%)	50 (11.7%)	50 (0.46%)	11 of 21 (52.4 %)
2003	23,977	1165 (4.85 %)	200 (17.2 %)	200 (0.83 %)	36 of 88 (40.9 %)
2004	24,803	1277 (5.15 %)	241 (18.9 %)	241 (0.97 %)	56 of 123 (45.5 %)
2005	23,087	1271 (5.51%)	223 (17.5%)	223 (0.97%)	None listed in CDR database

testing for *C. difficile*, as well as a statistically significant ($P < .01$) increase of 111% in the number of cases of *C. difficile* during the study period (Figure 1).

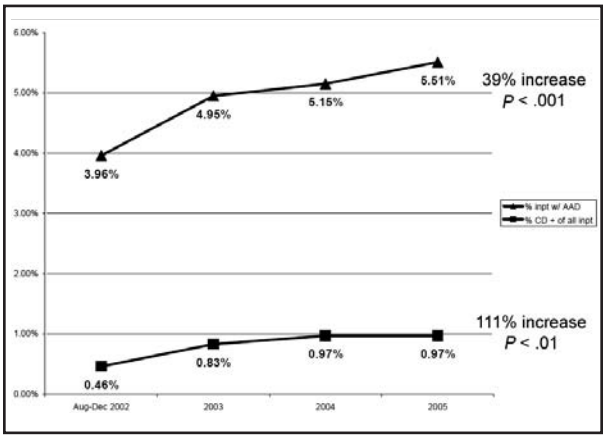

Figure 1. Diarrheal fecal specimens and Clostridium difficile infection (CDI) among inpatients at the University of Virginia Health System (UVA), 2002-2005. Line with triangles represents the percentage of UVA inpatients (inpt) with a diarrheal fecal specimen, considered a marker of antibiotic-associated diarrhea (AAD). Line with squares represents the percentage of UVA inpatients with CDI (CD+).

With the use of fecal lactoferrin as a marker of inflammation, overt intestinal inflammation was detected in nearly half of the hospitalized patients with CDI each year (see Table 1 for specific rates). With 40.9%-52.4% of patients having overt intestinal inflammation, data suggest not that there is a trend toward an increase in the number of diagnosed CDI cases but also that these cases remain comparably inflammatory as assessed by the presence of fecal lactoferrin, a potential marker of disease severity.

To determine the association of CDI with specific antibiotics, we focused on antibiotic use in 2004 and examined information on the 24,803 hospitalized inpatients. Of a total of 1992 inpatients (8%) on ceftriaxone, 293 (15%) were tested for *C. difficile*, and 61 (3%) were positive. Of a total of 1247 inpatients (5%) on clindamycin, 123 (10%) were tested for *C. difficile* and 15 (1%) were positive. Of the 2922 inpatients (12%) on ciprofloxacin, 629 (22%) were tested for *C. difficile* and 135 (5%) were positive (Table 2).

From 2002 to 2004, there was not an increase in the percentage of inpatients treated with ciprofloxacin (mean 11.51, SD 0.01). However, the percentage of inpatients receiving ciprofloxacin nearly doubled in 2005 (21.22%), but not in 2006 (12.59%). Starting in March 2002, moxifloxacin was the other fluoroquinolone on the UVA formulary. Moxifloxacin, however, accounts for only 15% of fluoroquinolone use. In our analysis, the percentage of patients on moxifloxacin who were tested for *C. difficile* (23%) was essentially equivalent to the percentage of patients on ciprofloxacin tested for *C. difficile* (22%). However, the rate of *C. difficile*–positive fecal specimens was actually higher with ciprofloxacin than moxifloxacin, 4.6% and 2.9%, respectively. This difference was statistically significant ($P =. 033$).

In 2004, ciprofloxacin was used more than ceftriaxone or clindamycin, and more than twice as many patients treated with ciprofloxacin were tested for *C. difficile*. The rate of *C. difficile* testing was 50% more with ciprofloxacin than ceftriaxone and more than twice that of clindamycin. The rate of CDI was 1.5 times more with ciprofloxacin than with ceftriaxone, and 3.8 times that of clindamycin. The absolute number of CDI cases associated with ciprofloxacin was more than 2 times that with ceftriaxone and 9 times that with clindamycin. Of those patients with CDI who were tested for fecal lactoferrin, nearly half in all groups had increased fecal levels. This results of this study suggest that ciprofloxacin is a major driver of CDI at our institution.

Table 2. Evaluation of Specific Antibiotics, 2004

	Patients Tested for C. difficile	C. difficile + of all Antibiotic-Treated Patients	C. difficile + Tested for Lactoferrin	Lactoferrin + of C. difficile Toxin +	
Ceftriaxone	1992 (8.03%)	293 (14.71%)	61 (20.82%)	35	19 (54.29%)
Clindamycin	1247 (5.03%)	123 (9.86%)	15 (12.20%)	7	3 (42.86%)
Ciprofloxacin	2922 (11.78%)	629 (21.53%)	135 (21.46%)	72	33 (45.83%)
Moxifloxacin	524 (2.11%)	118 (22.52%)	15 (12.71%)	0	0
Total no. patients treated (N = 24, 803)			1277	114	55 (48.25%)

DISCUSSION

CDI is a growing concern owing to increasing frequency and severity. Ciprofloxacin is one of the most widely used antibiotics with a broad spectrum of indications. At our institution, ciprofloxacin was the main fluoroquinolone used during the study period and was found to be more associated with CDI than to moxifloxacin. In 2006, Dhalla et al found no difference in the CDI rate associated with moxifloxacin compared with ciprofloxacin and other fluoroquinolones.[10]

A main limitation of this study is effect of the use of an existing database on the validity of the information. The prevalence estimates presented might be artificially low because our definition of CDI had only 1 criterion, a positive result for a *C. difficile* toxin assay performed in the UVA microbiology laboratory. The study is missing CDI cases that were diagnosed at outside institutions and cases that were not tested. The specificity of the data obtained, however, is likely quite high. The use of testing for *C. difficile* as a marker of antibiotic-associated diarrhea also contributes to potential bias. It is possible that a test could have been ordered on a patient who had not received antibiotics. Also, we have not accounted for patients who had diarrhea while on antibiotics but did not have a sample sent for testing. The results of a *C. difficile* toxin assay, although reported in textual format, are accurate, in that a positive report reflects a specific quantitative result. The data from the CDR did not account for the duration of antibiotic treatment, use of multiple antibiotics, length of stay, or disease severity.

The number of cases of CDI at our institution has steadily increased since 2002, although not to the extent of the increase reported in Québec. In 2004, 2992 (12%) of all inpatients received at least 1 dose of ciprofloxacin. Given the heavy presence of ciprofloxacin in clinical medicine, it is crucial to determine the rate of ciprofloxacin-associated *C. difficile*. Although the results of this study further support concerns that ciprofloxacin is a major potential driver of CDI, more definitive studies are needed.

Clinicians must closely follow their patients on antibiotics in order to rapidly diagnose CDI. In relation to the other antibiotic treatments we evaluated, ciprofloxacin treatment made patients 50% more likely to be tested for *C. difficile* than clindamycin treatment and more than twice more likely than ceftriaxone treatment. Ciprofloxacin was associated with 50% more positive stool tests for *C. difficile* than ceftriaxone and nearly twice as many positive tests as clindamycin. Nearly 5% of the patients given ciprofloxacin at our institution developed CDI. The absolute number of CDI cases associated with ciprofloxacin was more than 2 times the number associated with ceftriaxone and 9 times the number associated with clindamycin. Of CDI patients tested for lactoferrin in a study by Guerrant et al, nearly half in all groups showed overt intestinal inflammation.[11] Hence, increasing rates of CDI are associated with similar rates of intestinal inflammation, despite the antimicrobial agent used. This study highlights the relation of CDI with specific antibiotics and suggests that ciprofloxacin is potentially a major driver and cause of increasing CDI at our institution.

DISCLOSURES

This research was supported in part by NIH/NIAID U01 AI070491-01 and NIH T32 AI 55432-03. No author has any commercial or proprietary interest in the data or methods presented in this manuscript. Potential conflict of interest: Richard Guerrant has licensed fecal lactoferrin to TechLab, Blacksburg, VA. Other authors have no conflicts of interest.

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