A Case of Polyneuropathy and Proteinuria

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Introductory Remarks

Gerald B. Appel, MD: Welcome to the ASN 2006 CPC. The CPC is always a major challenge. The CPC is not to see if the clinician gets the answer right. It’s his thought process that counts—to see if his thought process matches your thought process.

We have the right people here today. We have two colleagues who will be doing this CPC today. First, Dr. Steve Korbet from Rush University Medical Center in Chicago, our clinician, who we will put to the test to see his clinical smarts and thought process. Working hand in hand with him, we have Dr. Steve Bonsib from Indiana University. So let’s get started with the Midwestern group of presenters we have here.

Steve Korbet: It’s a pleasure to be a part of this CPC. Dr. Bonsib and I have a little history. I have been involved in doing CPC-like vignettes with him at previous ASNs and at his institution as a visiting professor in Indianapolis. My diagnostic success rate has not been too good, one for seven I believe, and I’m sure today will be no different. Dr. Bonsib will no doubt prove again that the gift of the renal biopsy given to us some 50 yr ago by Iversen and Brun in Denmark and Kark and Muehrcke here in the United States is still as precious and valuable in managing patients and guiding therapy as it was 50 yr ago (1–3). I think that one thing we can count on, whether I get the case right or not, is that the clinical value of the renal biopsy remains immeasurable.

I was asked to do the CPC by Dr. Paul Kimmel some months ago. On August 15, 2006, I received an e-mail with the case which read, “Please call or write if you have any questions. Good luck,” and I thought, since this is coming from Washington, DC, it was possible Dr. Kimmel might not be able to express in writing everything he wanted, and maybe something was encrypted. So I had our chief research technician take the e-mail back to the lab to evaluate it further, and sure enough, an additional message was found: “Good luck. Because we’re not going to answer any questions you have.”

So, to get on to the case: The patient is a 66-yr-old Hispanic female, born and raised in the United States. The chief complaint is she’s dizzy, became lightheaded, and nearly fainted when getting out of her car. She has since restricted her activity although she has been treated in the past for urinary tract infections. She states that her health has overall been good. She specifically denied systemic symptoms, such as fever and chills, nausea, and vomiting but mentioned occasional bouts of diarrhea over the past few months. She has noted numbness and tingling in her upper and lower extremities and decreased sensation in her lower extremities as well. She has denied lower urinary tract symptoms, such as frequency, urgency, or pain on urination, and did not notice blood in her urine. She has not traveled outside of the United States and has no ill pets or recent insect bites.

She is on no medications whatsoever, we’re told. Her family history is important in that there is no known family history of renal disease. The patient’s father had congestive heart failure and was disabled. He was wheelchair-bound, because of an undiagnosed type of neuropathy. He died suddenly at age 58. There was no autopsy performed. Review of symptoms was negative, except for what was noted.

“On physical exam, her BP was 160/95. We’re not told if she’s orthostatic or not. Her heart rate is 72, her weight is 155 lb. The CBC demonstrated an anemia with a hemoglobin of 10.6 mg/dl, hematocrit of 31.4%, and the white count was 6.3 × 10^3/mm^3 with a normal differential and the platelet count was 103/mm^3 with a normal differential and the platelet count was

We were given a host of laboratory data. Her sodium was 140, potassium 5.1, chloride was 107, and bicarb was low at 21. The anion gap was 12. We’re not given a blood gas pH, or a Pco2, but I’m going to just assume that maybe she has a metabolic acidosis, nonanion gap in nature. The BUN was 48 and creatinine was 3.6 mg/dl. If you estimate her GFR using the MDRD formula, assuming this represents chronic kidney disease (CKD), and I don’t know that for a fact, you get an estimated GFR of about 14 ml/min, which would put her in category 5 CKD. Her fasting glucose was 115 mg/dl, and we were only given one result for this. We were not given any hemoglobin A1C values. The calcium (9.4 mg/dl), albumin (4.4 g/dl), AST, and ALT were normal, but her alkaline phosphatase was elevated.

The CBC demonstrated an anemia with a hemoglobin of 10.6 mg/dl, hematocrit of 31.4%, and the white count was 6.3 × 10^3/mm^3 with a normal differential and the platelet count was
225,000/mm³. The urinalysis had 1- to 2-plus protein with no red cells, white cells, or casts, and thus she had no abnormal urinary sediment. A 24-h urinary protein excretion was 1200 mg/d. Now we don’t know what kind of protein that was. We don’t know whether this represents glomerular or tubular proteinuria, it could be either one.

Serologic examination shows that she had a C3 and a C4, which were normal, and the evaluation for ANA, hepatitis B and C, and HIV were negative. We’re given no indication that there was an assessment for ANCAs. A serum protein electrophoresis demonstrated a small amount of free λ light chains. No indication is given that an assessment for a paraprotein in the urine was performed.

Chest x-rays demonstrated cardiomegaly. CT scan demonstrated a small amount of pelvic fluid and mild pericardial and bilateral pleural effusions. The kidneys were normal in size with no hydronephrosis and no stones based on an ultrasound evaluation. We’re not told what the echogenicity is. A renal biopsy was performed.

So, we’ve got a lot of stuff on our table here, and I tried to summarize it in this way (Table 1). We have a 66-yr-old lady who presented with dizziness, who, in fact, had many symptoms and signs of polyneuropathy. Polyneuropathy, by definition, implies peripheral nerve disease, which is bilateral, symmetric, and is sensory as well as motor. It usually occurs distally first, and often the sensory manifestations precede the motor, and so she has a classic polyneuropathy with distal sensory deficits as her primary finding, involving both lower and upper extremities.

She also has diarrhea, and I’m assuming this may be as a result of a coexisting autonomic neuropathy. She has evidence of a cardiomyopathy as she has an S3 on her physical exam and an enlarged heart on x-ray.

She may or may not have a hepatopathy. I don’t know what to make of the alkaline phosphatase, it’s definitely elevated. I don’t know if it’s elevated because of something going on in the liver or whether it’s elevated because she has secondary hyperparathyroidism from chronic renal disease. We’re not given a phosphate, we don’t know what her PTH is, so kind of broadening things out here, I’ll assume that may be a subtle clue that something is going on in the liver.

She definitely has a nephropathy. She has what may be a type 4 RTA, with a high-normal serum potassium and low bicarb level associated with a normal anion gap. She has proteinuria, which could be tubular or glomerular in nature, we just don’t know, but the presence of both low-grade proteinuria and the type 4 RTA is suggestive of a tubulointerstitial process.

And I think she probably has CKD. She doesn’t have an active urine sediment, and there doesn’t seem to be anything acute going on to account for her renal insufficiency. Thus, I think this may be something that’s more chronic than acute, but interestingly enough, her kidneys are normal in size, something you wouldn’t expect in someone with CKD per se, but there are a few conditions that can be associated with normal kidney size despite chronic renal disease.

Finally, she has free λ light chains. Now I don’t know if this is something that’s to be taken seriously or if this is just being thrown out there to confuse me. A number of years ago, Dr. Agnes Fogo showed that in the evaluation of patients with paraproteins, the renal biopsy findings are unrelated in over 60% of cases (4), so I don’t know if they’re just baiting me a little bit, but this is what we have. In essence, we have a patient who has a multisystem disorder (Table 1). Based on all of these findings, I came up with this laundry list of diagnoses that are possible, and I hope to God one of them is right!

The differential diagnosis includes (Table 2) metabolic diseases, granulomatous and autoimmune diseases, and disorders associated with paraproteinemias or plasma cell dyscrasias. What I’m going to do is try to briefly go through these disorders and see which one will fit best.

Patients with type 2 diabetes mellitus will have a polyneuropathy, especially after they’ve had their diabetes for about 10 yr, and it can present just as this lady has presented: Distal, sensory before motor, in a stocking-glove pattern. Diabetics can definitely have diarrhea from a neuropathy. They can have a cardiomyopathy from small-vessel disease, as well as coronary artery disease. They can have a hepatopathy from steatosis and a nephropathy associated with a type 4 RTA, proteinuria, and advanced renal disease, and this is one of the settings where you can have normal-sized kidneys despite advanced renal disease, but they shouldn’t have λ light chains. The bigger problem here, you’re all probably saying, is that she’s not diabetic, and so it’s kind of hard to make that a serious diagnosis unless this is someone who has had “subclinical” or undiagnosed chronic hyperglycemia, but I can’t believe they’d be that unfair and not provide me with a fasting glucose that was elevated or an abnormal hemoglobin A1C, but we’ve all

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**Table 1. Clinical features**

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<tr>
<th>Neuropathy</th>
<th>polyneuropathy (distal sensory defects)</th>
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<tr>
<td></td>
<td>autonomic neuropathy (diarrhea)</td>
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<td>Cardiomyopathy</td>
<td>(S3 and cardiomegaly)</td>
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<td>Hepatopathy</td>
<td>(elevated alkaline phosphatase)</td>
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<td>Nephropathy</td>
<td>type 4 RTA</td>
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<td></td>
<td>proteinuria</td>
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<td>CKD stage 5</td>
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<td>normal-sized kidneys</td>
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<td>Paraproteinemia</td>
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**Table 2. Differential diagnoses**

1. Diabetes mellitus
2. Fabry’s disease
3. Sarcoidosis
4. Systemic lupus erythematosus
5. Wegener’s granulomatosis
6. POEMS syndrome
7. Light-chain deposition disease
8. Primary (AL) amyloidosis
seen patients who have been followed with chronic “mild” elevations in their glucose and have been told, “Don’t worry about it.” Ultimately, this has resulted in type 2 diabetes that’s gone untreated for years, and then they present with end-organ damage, but I don’t think this lady is going to have diabetes.

What about Fabry’s disease? Fabry’s disease is an X-linked recessive disorder resulting from a deficiency of α galactosidase. This can lead to a polyneuropathy, but the neuropathy in Fabry’s disease is usually extremely painful and is brought on by cold as well as stress. These patients do get diarrhea. They can also have a cardiomyopathy from the vasculopathy that they get, which can lead to LVH, coronary artery disease, as well as CHF. They usually don’t get a hepatic disease. They get a nephropathy that’s progressive in nature, and by the age of 55, essentially 100% patients, especially males, will be on dialysis. The kidney size may be normal, but you shouldn’t expect to see a light chains. Granted, women, especially, will have a variable presentation because they’re most often hemizygous.

We’re told there is no family history of renal disease, and the only family member we’re given specific information about is the father, who died at age 58. If he was the carrier, he should have had ESRD and didn’t.

Additionally, the patient doesn’t have any evidence of skin manifestations. I would have thought that anyone with such multiorgan disease due to Fabry’s would also have dermatologic features: Telangiectasias or angiokeratomas. So, the fact that she doesn’t have any skin lesions and, most important, that there is no family history of renal disease, knocks Fabry’s out of the box for me.

What about sarcoidosis? Sarcoidosis is associated with neurologic abnormalities, but the neuropathy is mononeuritis multiplex, which is not symmetric as is a polyneuropathy. The predominant neurologic manifestations in sarcoidosis are result of granulomatous disease involving the CNS, resulting in seizures and facial palsies due to cranial nerve involvement. They can have a cardiomyopathy, which leads to arrhythmias and heart failure, and often have a hepatopathy, which may present with an elevated alkaline phosphatase. While about 20% of patients will have hepatomegaly, 75% will have hepatic granulomas.

The nephropathy in sarcoidosis is frequently tubulointerstitial in nature, leading to chronic interstitial nephritis. Additionally, they can have renal disease secondary to hypercalcemia and hypercalciuria, leading to nephrocalcinosis, and in some cases, they may have glomerular disease with membranous glomerulopathy or FSGS.

Now, if somebody had sarcoidosis that had as much systemic involvement as our patient, I would expect that they would also have pulmonary or skin disease, and she doesn’t. Additionally, if they had this much of a granulomatous load, I would have expected the patient to have some mild hypercalcemia or calcification, and we’re not given any indication that she’s ever had that either. Patients with sarcoidosis should not have monoclonal λ light chains, so I’m taking sarcoid off the list as well.

What about systemic lupus erythematosus? Patients with lupus can have neurologic manifestations such as transverse myelitis, and they can occasionally have a polyneuropathy in a stocking-glove-type distribution. They don’t usually get diarrhea but can have cardiomyopathy from coronary vasculitis or valvular disorders. They usually don’t have hepatopathy, and patients previously diagnosed with “lupoid hepatitis” now are recognized as having chronic active hepatitis. They frequently have a nephropathy, at least the patients we see, often glomerular in nature, but these patients could have tubulointerstitial involvement as well, leading to RTA and lesser degrees of proteinuria and progressive renal failure.

I wouldn’t have expected the kidney size to be normal in a lupus patient with advanced renal insufficiency. I would have thought that if they were giving me a lupus patient with such widespread involvement and lupus nephritis, the patient should have an active urinary sediment, and this patient had no abnormal sediment whatsoever, and again, while patients with lupus may have a polyclonal hypergammaglobulinemia, they shouldn’t have free light chains, if the light chains are really important here. I think the other big issue is the lupus serology was negative in this patient. For numerous reasons, I think it is unlikely our patient has lupus.

What about Wegener’s? Wegener’s patients too have a multisystem disorder. About 15 to 20% of patients with Wegener’s may have a neurologic manifestation as the presenting feature. They often have mononeuritis multiplex and not a polyneuropathy. They can also have cranial nerve involvement, and diarrhea isn’t common. They can definitely get a cardiomyopathy from pericarditis, coronary artery vasculitis, or pancarditis. Hepatopathy is not usually seen. They, too, like lupus patients, frequently have an active glomerular process due to a small-vessel vasculitis and can have tubulointerstitial involvement, too. They should not have free λ light chains. I would have thought if somebody had Wegener’s, with all these other organ systems involved, that they would at least have the more common features, such as pulmonary and upper respiratory tract disease, but this patient doesn’t have any evidence of that.

They gave us no serology whatsoever; in fact, they didn’t even mention the ANCA. So I don’t think they’re going to make this a Wegener’s case.

What about POEMS? Well, POEMS is defined by polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes (5). This disorder is due to an upregulation of proinflammatory cytokines, usually VEGF. These patients will have a polyneuropathy, which primarily involves the lower extremities and presents with distal paresthesias and progressive weakness, similar to our patient; however, they don’t have an autonomic neuropathy, with diarrhea. The organomegaly includes hepatomegaly, splenomegaly, and they can have a lymphadenopathy, features not seen in this case.

Endocrinopathies, pick one, they can have hypothyroidism, diabetes, hypogonadism, hypopituitarism. This patient doesn’t have any of these. A monoclonal protein, usually λ, is seen in about 85% of cases. Essentially, all patients will have skin changes with hyperpigmentation and hemangioma. Renal and cardiac involvement are each seen in 40% of cases. Even though a number of features in our case would suggest PEOMS syndrome, the lack of skin lesions, organomegaly, or endocrinop-
athy makes it difficult for me to believe that this is the diagnosis.

Light chain deposition disease (LCDD) is a possibility. One of the largest series from Italy by Pozzi et al. (6) describes their experience in 63 patients. The average age at presentation is 58 yr, just about right for our lady who is in her mid-60s. Proteinuria over 1 g/d is seen in 84% of these patients. The majority (over 90%) have renal insufficiency at presentation. A paraprotein, most often free κ light chains, is found by immunofixation of serum or urine in 94% of cases, and about 65% of patients have multiple myeloma. Extrarenal disease is observed in 35% patients, and cardiac involvement is most common (21%) followed by liver (19%) and neurologic disease (8%). So, in my mind, the diagnosis of LCDD is a strong possibility with the exception that our patient had λ, as opposed to κ, light chains, but a third of LCDD patients can have λ light chains.

What about AL-amyloid? The features for AL-amyloidosis are essentially the same as in LCDD. AL-amyloid is a disease of older adults, and proteinuria is often a presenting finding along with renal insufficiency (7). A paraprotein is found in over 90% of cases and is usually λ in 70% of cases, not unlike our patient. Multiple myeloma is less often seen, about 21% of cases. These patients, as is well known, also have heart disease, peripheral neuropathy, and can have hepatopathy (often heralded by an elevated alkaline phosphatase).

One other thing to keep in mind is this patient didn’t have a lot of proteinuria, and we usually think of patients with LCDD and AL-amyloid as being more proteinuric and ultimately developing the nephrotic syndrome. In a small proportion of patients with AL-amyloidosis, renal failure can be the predominant finding and may be associated with minimal or no proteinuria. The renal biopsy in these patients demonstrates predominantly vascular and interstitial amyloid with essentially no glomerular involvement, so I think amyloidosis is still in the differential.

So LCDD and AL-amyloidosis remain possibilities in this case, and it may be just the toss of a coin to decide which one. There’s just one problem. There’s an 800-lb gorilla in this case that I just can’t ignore, and that’s the father who died with disabling neuropathy and cardiomyopathy. He never had renal disease, but the fact that the father had a neuropathy that was so disabling that he became wheelchair-bound, had congestive heart failure, and then died suddenly before a diagnosis could be made doesn’t minimize the potential that he is somehow important to this case, especially given the fact that his daughter has a neuropathy as her primary presenting feature and also has a cardiomyopathy. So I think this patient may actually have a hereditary form of polyneuropathy, and that would eliminate my two remaining diagnoses (LCDD and AL-amyloidosis), so now I’m really in trouble.

If you look at the laundry list of hereditary polynueropathies and the other organ systems involved in many of these disorders, there are only two that have kidney disease associated with them: Fabry’s disease and hereditary amyloid polyneuropathy. Now we’ve already ruled out Fabry’s, and what is left is the possibility that this patient may have a form of hereditary amyloid polyneuropathy with involvement of the heart and kidney. In an excellent review of their experience, Lachmann et al. (8) found almost 10% of 350 patients previously thought to have primary amyloid and having no family history of amyloid actually had a familial form of amyloidosis when genetic analysis was performed. Hereditary amyloid is inherited as an autosomal dominant trait with variable penetrance. Mutations in fibrinogen-α chain, apolipoproteins 1 and 2, lysozyme, and transthyretin are the most common causes. Interestingly enough, mutations in fibrinogen-α chain, apolipoproteins 1 and 2, and lysozyme are primarily associated with a renal amyloid presentation or have a non-neuropathic presentation, while those associated with transthyretin usually present with a polyneuropathy as the primary feature.

Unlike primary amyloidosis, these patients will present from as early as the second decade of life up to the eighth but are otherwise clinically indistinguishable from AL-amyloid. They can have an associated paraprotein 24% of the time, much less than you would see in AL-amyloid, and their course is more indolent than that of AL-amyloid. The real issue here is that it’s critical to make the diagnosis of hereditary amyloidosis as the treatment is liver transplantation and not chemotherapy (9,10).

Mutations in fibrinogen-α chain were observed in 18 of 34 patients with hereditary amyloid reported by Lachmann et al. (8). The median age in these patients was about 60 yr. Twenty-two percent had a low-grade paraproteinemia. Hypertension was common. They presented with renal insufficiency and proteinuria, and two thirds of them progressed to ESRD over about 2 yr, but none of them had cardiac disease or neuropathy, so it makes this mutation, even though it was one of the most common ones found, less likely in our patient, if in fact this is what our patient has.

When Lachmann et al. (8) looked for patients with transthyretin mutations, and there are apparently over 80 mutations in transthyretin, they identified 13 patients. Transthyretin is a carrier protein for thyroid hormone; it’s a tetramer, but mutations in transthyretin cause it to dissociate into monomers, which can then polymerize and form amyloid (11). In the series of Lachmann et al. (8), the median age was 62 yr of age, which was similar to our patient. All presented with peripheral and autonomic neuropathy. All had cardiac amyloid, and, in fact, many of these patients can die with their cardiac amyloid before their renal disease is actually diagnosed. Twenty-five percent had low-grade paraproteinemia, and six ultimately had evidence of renal involvement.

In a large series from Japan, of 169 patients with transthyretin-related familial amyloidotic polyneuropathy (FAP), the Val30Met mutation (methionine replacing valine in position 30) was the most common form (12). The mean age at diagnosis was 35 yr of age in this study, but this can vary between countries. They present with sensory motor neuropathy usually involving the lower limbs, and they had an autonomic involvement. Additionally, cardiac involvement, resulting in conduction abnormalities and cardiomyopathy, and renal involvement were common. Transthyretin-related FAP progresses more slowly than AL-amyloid (which historically has a life expectancy of 1 to 2 yr), leading to death within 5 to 15 yr (10–12). The most common locations where patients with transthyre-
tin-related FAP with the Val30Met mutation have been described are Japan, Sweden, and Portugal. Interestingly, the disease presents at an earlier age in reports of patients from Japan and Portugal than those from Sweden. Now we were told that this patient is Hispanic, but I have found that the term Hispanic is one that was given by the Romans to the inhabitants of the Iberian Peninsula and that somewhere around 1640 that term was reserved for Spanish people and not for Portuguese.

The term Lusitanic is now used to refer to people of Portuguese culture or descent. In our patient, I'm not certain what the term Hispanic implies but could mean that she is of Portuguese descent as we are not always as precise with this terminology in this country, but suffice it to say, when I looked at a world map that noted the various locations where this mutation has been reported, I found a dot right over the state of Indiana, and knowing that Dr. Bonsib comes from Indiana (and I assume our patient did too), my feeling is that somebody from Indiana has obviously been reported with this mutation, so people from Indiana, possibly non-Portuguese, non-Japanese, non-Swedish, can have this disorder as well—I'm hoping.

The presenting clinical symptoms include—and our patient had a large number of them—sensory disturbances in the lower limbs (44%), gastrointestinal symptoms (32%), faintness or syncope (4%), proteinuria (4%), muscle weakness in the lower limbs (3%), and arrhythmias (4%, which may have led to our patient's dizziness). Ultimately, the majority of patients have symptoms, such as dysesthesias, muscle atrophy, diarrhea, orthostatic hypotension, and arrhythmias, related to progressive involvement of peripheral and autonomic nerves, and cardiac involvement, and a small proportion will have clinical evidence of kidney disease (10%). Many of the symptoms, manifest in our patient (dysesthesias, sensory disturbances, and diarrhea) and in her father (cardiomyopathy and wheelchair bound due to a neuropathy), can be seen in this FAP.

So the diagnosis of transthyretin-related FAP fits well with our patient. Additionally, the renal disease that's been described in patients with transthyretin-related FAP is also consistent with that seen in our patient. While around 10% of cases have clinical evidence of renal disease, Labato et al. (13) performed renal biopsies in 14 patients that were going to have liver transplants and found histologic evidence of renal amyloid in all of the patients. The location of the deposits was predominantly tubulointerstitial, but there were some mesangial deposits. Proteinuria was demonstrated in seven patients, and in four patients it ranged from 0.3 to 4 g, while the remaining three patients had only microalbuminuria. They found that patients with overt proteinuria were more likely to have glomerular and vascular deposits, which were more extensive. Nonetheless, even patients with minimal proteinuria can develop progressive renal insufficiency from extensive tubulointerstitial disease (14). Finally, the renal involvement may be a late occurrence in patients with transthyretin-related FAP, which would explain why the father may not have had evidence of renal disease by the time of his death.

In conclusion, as one of my favorite characters, Sherlock Holmes, says, “Eliminate all other factors, and the one which remains must be the truth” (from Arthur Conan Doyle’s novel The Sign of the Four). After eliminating all of the other possibilities, I am left with the diagnosis of hereditary amyloidosis, transthyretin-related familial amyloidotic polyneuropathy—if that's not a mouthful, to be specific—and I will end there. Thank you so much.

Gerald Appel: Well, Steve, that may be brilliant. It may be brilliant, but you could be wrong. You never can tell with these CPCs. Steve, would you care to speculate which amino acid was replaced for which amino acid on this case? You have given us a pretty specific diagnosis. If this person were at Columbia, you would say, “This is a diabetic. This is one of the six million diabetics who came in and just happen to have a normal sugar.” Now, to get the truth, and so Steve can finally find out whether he’s brilliant or not, Dr. Steve Bonsib, our pathologist, will tell us the answer here.

Steve Bonsib: Thank you. Steve exaggerated his inaccuracy rate on our CPCs. It’s more like six for seven correct, rather than one for seven. Just to provide encouragement when we were putting this CPC together, he was kind enough to send me an initial PowerPoint presentation of his talk, so if he got the diagnosis correct, we would make certain that we didn’t duplicate the content too much. To provide positive support, my response was, “Boy are you going to be embarrassed.”

I have always enjoyed the CPC because it takes a courageous individual to stand up and bare their innermost thought processes for all to see; however, after they dissect the clinical history, they usually end up with the right diagnosis. Often, along the way, they point out the limitations of the poor pathologist who is trying to craft the clinical history. Steve was kind enough not to point out too many flaws in the clinical history.

Nephropathologists pride themselves in their clinical acumen, such as it is. I did struggle to put this clinical history together in a complete form, but when you look at these charts, there are so many words and numbers, it just doesn’t compare with the esthetic art of a nicely prepared glass slide. I found that the harder I looked at this case, the less I saw (Figure 1), but, in
the final analysis, I knew Steve would have a real good chance of overcoming the limitations of my clinical history and get the right diagnosis anyway.

It can be similarly amusing when clinicians venture into the domain of the pathologist. I remember a number of times walking into a sign-out room and watching a clinician gripping a glass slide with two hands, trying with great difficulty to find a section, much less a glomerulus, and keep it in focus. Even more amusing is when I’d go into the sign-out room and I’d find a group of clinicians trying to make a diagnosis “under the microscope” (Figure 2).

Fortunately, when the pathologist and the nephrologist get together, there’s a synergism that does great good for our patients; the CPC epitomizes that process. Well, I think it’s time we actually take a look at the biopsy and see if my confidence in Steve is well placed.

Here’s the patient’s biopsy. As you can see, there are two cores of tissues. The first core is mostly cortex with a little bit of outer medulla (Figure 3). The second one is mostly outer medulla with a little bit of cortex. If we look at this region of cortex first, there’s a thickened sclerotic artery and two sclerotic glomeruli. Here’s one of those glomeruli on PAS stain. There is nothing unusual about the pattern of sclerosis, simply arterial nephrosclerotic changes.

We do have a couple of nonsclerotic glomeruli (Figure 3). Let’s look at them more closely. On H&E stain, there is no particularly striking abnormality; similarly, on PAS and silver stain, we can only see mild mesangial changes (Figure 4). The capillary loops are a little wrinkled and starting to collapse, but no significant glomerular abnormalities are present. This correlates with the low-grade proteinuria; however, I’m sure you’ve noticed that there is an impressive degree of tubulointerstitial disease with extensive interstitial expansion (Figure 3). If we look at the interstitial areas at higher magnification, we see only a few tubules that are all undergoing atrophy. Of note, the interstitium has extensive lumpy eosinophilic deposits that on silver stain are weakly argyrophilic, or stain lightly with the silver stain, a distinctive finding for this material (Figure 5).

On immunofluorescence, the glomeruli, vessels, and interstitium were negative for all immunoglobulins, complements, and both light chains. The κ and λ stains only stained protein casts. There are no significant immunohistochemical findings on direct immunofluorescence.

Looking at electron microscopy, here is a portion of a glomerulus with capillary loops, the urinary space, capillary loop basement membranes, and mesangial matrix. There is focal effacement of podocyte foot processes, with many foot processes remaining. The capillary loop basement membranes are
slightly thickened and a little wrinkled but contain no obvious deposits. The mesangial matrix is slightly expanded, but there’s no evidence of electron-dense deposits or paraprotein deposits. If you look at high magnification of the mesangial matrix, you can see the mesangial microfibrillar substructure, normal for the mesangium.

If we direct our attention to the area that was most abnormal by light microscopy, the interstitium, we can see several interstitial cells and the lumpy deposits that at high magnification have the delicate fibrillar random array appearance, characteristic of amyloid fibrils (Figure 6).

To confirm that this material is amyloid, here is a Congo red stain. Unfortunately, I can’t photograph a good polarized apple-green birefringence with my camera, but it did show apple-green birefringence. Because of the neuropathy and the clinical concern of a familial disease, immunoperoxidase stain was performed for a transthyretin. It is positive, as you can see, with an appropriate negative control (Figure 7); therefore, the final diagnosis is a hereditary amyloidosis, familial amyloid polyneuropathy secondary to transthyretin-associated amyloid. Dr. Korbet was right on the mark, as I expected. What a relief.

There have been at least 21 amyloidotic proteins identified, and more are yet be identified (15). AL-amyloid is the most common type, followed by AA-amyloid as second most common. The hereditary amyloid syndromes, collectively, are the third most common form of system amyloid. Thus, this is a group of diseases that’s very important to consider in a patient with amyloidosis.

This is a list of the familial amyloid syndromes; there are seven (Table 3). The first one listed, transthyretin, is the most common member of this group that also includes apolipoprotein A1 and A2, cystatin C, lysozyme, fibrinogen-Aα chain, and gelsolin (12,15–20). Most familial forms of amyloid result from a single amino acid substitution. All are autosomal dominant, and most present after the age of 50. If we look at their geographic occurrence, there are four that are most common in the United States: Transthyretin, apolipoprotein A1 and 2, and fibrinogen-Aα chain (12). Others occur in other geographic ranges.

Let’s focus on transthyretin-associated amyloid; it’s important to remember that there are two forms. There’s a nonfamil-
ial form, known as senile cardiac amyloid, although patients also have amyloid outside the heart (21). This is the most common form of amyloid, although it’s often not symptomatic. In this condition, the wild-type protein is deposited, not mutated protein. If one looks carefully at autopsies in patients over the age of 80, you can find it in as many as 20 to 25% of cases. Occasionally, senile cardiac amyloid will cause heart failure and other symptoms. This is an example of an autopsy case from a few years ago with cardiac amyloid that was believed to be senile cardiac (Figure 8). I say believed because molecular analysis of the amyloid was not performed.

The case discussed today is a case of familial amyloid polyneuropathy; in this disease, a mutated protein is deposited. Over 100 mutations of transthyretin have been identified—90% form amyloid (12,15). Because of the large number of mutations, the clinical phenotype and the pattern of tissue deposition can vary somewhat, although certain generalizations can be made. The onset of disease in most patients is after the age of 50. They have neuropathies, restrictive cardiomyopathy, the usual cause of death, and renal disease in 50% of patients. There are other clinical phenotypes. For instance, there is an early-onset form, due to a mutation of Val30Met that occurs in Northern Portugal (13,22,23). In these individuals, the onset can be quite a bit earlier, by a couple of decades.

In addition to the clinical spectrum, there’s also a morphologic spectrum. Renal involvement occurs in 50% of patients, but the location of the deposits may vary. Steve has already mentioned that often medullary interstitial or, as in this case, cortical interstitial deposition may predominate or may be the only site of involvement (13,15,23). Glomerular and vascular involvement may occur, that can be mild or marked. Thus, the renal manifestations can vary from renal insufficiency, with little or no proteinuria, to overt nephrotic syndrome, which we usually expect in amyloidosis, rather than just renal failure.

Here are two additional cases of transthyretin-associated amyloid that I will briefly illustrate. If you look at the paraffin blocks that we generate our slides from, shown here, you can see orange discoloration of the renal medulla. There was enough amyloid deposited in the renal medulla that you can actually see it grossly in the paraffin blocks. In this first case, there was no cortical involvement. The glomeruli are completely normal; there is no interstitial or vascular involvement (Figure 9A). All of the amyloid is in the renal medulla, this pale material staining on silver stain.

### Table 3. Familial amyloidosis: Geographic occurrence and nature of renal involvement

<table>
<thead>
<tr>
<th>Amyloid Type</th>
<th>Abbreviation</th>
<th>Geographic Incidence</th>
<th>Sites of Renal Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transthyretin</td>
<td>ATTR</td>
<td>Worldwide</td>
<td>Int, occ glom</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>ApoAI</td>
<td>US, Europe</td>
<td>Int + / − glom</td>
</tr>
<tr>
<td>Apolipoprotein A-II</td>
<td>ApoAll</td>
<td>US, Russia</td>
<td>Glom, vasc</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>ACys</td>
<td>Iceland</td>
<td>Usually none</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>ALys</td>
<td>Canada, Europe</td>
<td>Glom, vasc, int</td>
</tr>
<tr>
<td>Fibrinogen-Aα</td>
<td>AFib</td>
<td>US, Europe</td>
<td>Glom</td>
</tr>
<tr>
<td>Gelsolin</td>
<td>AGel</td>
<td>Finland</td>
<td>Mild glom</td>
</tr>
</tbody>
</table>

*int, interstitial; glom, glomerular; vasc, vascular.*
thioflavin T fluorescence stain for amyloid shows that the medulla was replaced by amyloid (Figure 9B).

The second case is very similar. Again, on thioflavin T stain for amyloid, we have the renal medulla with medullary collecting ducts; the interstitium is filled with amyloid deposits. An additional interesting aspect of this case is that the renal sinus arteries are negative for amyloid; however, the sinus veins are packed with amyloid, the converse of the usual pattern of vascular involvement in amyloid. Amyloid can occur in unexpected locations.

Today much greater responsibility rests upon pathologists than simply establishing a diagnosis of amyloid. I’d like to offer a diagnostic sequence of four steps that we should consider in amyloid cases; in some cases, we may have to go through all four steps. The first step is to recognize the presence of the abnormal material in the biopsy. The second step is to prove that the deposited material is amyloid. The third step is to characterize the amyloidotic protein precursor. The fourth step is to perform DNA or protein sequencing, if immunostains of step 3 are negative or equivocal. I’d like to take you through these four steps in greater detail.

First, recognize the presence of abnormal material. This is usually easy with amyloid. We’ve all seen many cases. Usually the glomeruli are replaced by amyloid. It’s a diagnosis a first-year pathology resident or a first-year nephrology fellow can make without a problem; however, there are more subtle versions, more challenging situations. The challenging situations are when there are mild degrees of glomerular involvement that can be overlooked or when we approach a case with a bias toward only identifying glomerular involvement. What’s nice about renal biopsy stains is that the silver stain is extremely useful in identifying small quantities of amyloid; I’d like to show you two examples. On the left we have a glomerulus. You can see that there are small acellular eosinophilic areas; based upon this talk, you’re going to think is amyloid, and it is. In this example of a modest degree of glomerular involvement by amyloid, note that the material is silver negative, a clue that the material deposited is amyloid and is not some other cause of mesangial expansion.

If you look at the glomerulus on the right, the amyloid is even more subtle. To my eye, this glomerulus looks completely normal on H&E stain. Yet on silver stain, notice we have little spicular arrays extending through two capillary loop basement membranes (Figure 10). Amyloid can be argyrophilic, particularly when in a spicular pattern. In this case, the subtle degrees of amyloid involvement are visible only on silver stain.

The other challenge is the bias toward recognizing glomerular involvement. Some cases of amyloid will be restricted to the vascular tree or interstitium. This example is predominantly arterial, although there is a little glomerular amyloid. If amyloid is completely arterial, there is a possibility that a patholo-
gist could regard the change as arterial sclerosis and not recognize it’s amyloid.

When amyloid occurs as pure medullary involvement, this can be a problem because nephrologists don’t normally try to sample the renal medulla. If you consider the number of patients whose biopsies contain only cortical tissue, you could certainly envision the possibility of occasionally missing the diagnosis simply because medulla was not represented. In familial amyloid syndromes, there are two types in which interstitial involvement, medullary and/or cortical, is a particular common finding: Transthyretin and apolipoprotein A1 (Table 3) (13,15,16,23).

Once we recognize the abnormal material, proving that it’s amyloid is usually straightforward. The gold standard is the Congo red stain viewed under polarized microscopy. The thioflavin T stain is a very sensitive fluorescence stain for amyloid, although maybe not quite as specific as a Congo red. Electron microscopy shows the characteristic fibular structure. If we only recognized amyloid on EM, we should confirm that it is amyloid with a follow-up Congo red stain.

Once we know the material is amyloid, we have to characterize the protein precursor because the therapy of these diseases is so different. We’ve already seen an immunoperoxidase stain in our index case of transthyretin-associated familial amyloid. Immunoperoxidase stain for AA protein is also a very reliable stain. Here’s an example of a rheumatoid arthritic patient with AA-amyloid deposited in the glomeruli (Figure 11).

The problem comes with the diagnosis of AL-associated amyloid. Here is an example of AL-amyloid with the A stain on the right and the corresponding H&E on the left (Figure 12). From 30 to 50% of light chain–associated amyloid will be negative on fluorescence stains because the antigenic moiety may be inactive or not present in the amyloid deposits (24). This is a significant problem since AL-amyloid is the most common type of amyloid.

In a recent publication by Kebbel and Röcken (25), a study of 121 patients with 169 biopsies from multiple locations, AL-amyloid was most frequent, AA second, and familial forms of amyloid were third most common. Of note, their immunostains were negative in 15% of the cases. They made the assumption that these 15% of patients were likely AL-associated amyloid. Although they went to great effort to identify several hereditary forms of amyloid, they were still left with a significant number that are negative by any of immunoperoxidase stains. The assumption often made is that these are AL associated; however, this may not be a good idea.

When immunohistochemistry stains are negative, we should consider molecular testing. We’re fortunate at Indiana University because we have Dr. Merrill Benson, who can do molecular studies easily. He can take a little piece from a heart biopsy or a little piece from a kidney biopsy and sequence the actual protein. Not only can he identify the actual protein, he can identify the specific mutation of that protein, which can be important in some of the familial syndromes. The molecular approach also allows us to identify new amyloidotic proteins.

We’ve already heard of the problem with making presumptive clinical diagnosis of AL-associated amyloid without immunohistochemical confirmation. Dr. Korbet mentioned this study of Lachmann et al. (9), in which 10% of their presumed AL-amyloid patients had a familial form of amyloid. As we know, in AL-amyloid, the usual treatment is chemotherapy or bone marrow transplant, which would not be the preferred treatment for a familial form of amyloid. In AA-amyloid, control of the inflammatory process is the main strategy. In the familial forms of amyloid, the therapy varies with the specific protein, thus the importance of knowing the identity of the protein involved.

The clinical evolution of many familial forms of amyloid here is slowly progressive, so supportive therapy, with or without a renal transplant, will be sufficient treatment. In some patients with fibrinogen-associated amyloid, the AFib variant
Glut526Val mutation, the course is much more rapid (20). These patients should be considered for liver transplant. For most patients with transthyretin-associated amyloid, liver transplant is the recommended treatment; however, not for all (15). With several transthyretin mutations, liver transplant is contraindicated because they will develop progressive cardiomyopathy and neuropathy from deposition of wild-type nonmutated protein on the preexisting mutated protein (26); therefore, not only is knowledge of the specific protein important, but also the specific mutation appears to be important.

On that note, I will end my talk. I’d like to acknowledge Cindy Nast, who provided the case that was discussed today, and Merrill Benson, who provided some examples of familial amyloid to photograph and for several informative discussions. Finally, I’d like to acknowledge my surrogate clinicians who are junior pathology residents that I bullied into sacrificing their dignity for the photographic spoof. Thank you very much.

Questions and Answers

Gerald Appel: You know, I’m not sure how many of you got the answer correct, but the one question I would have, and this is probably crucial to all this, is that how many centers can do this answer correct, but the one question I would have, and this is probably crucial to all this, is that how many centers can do this kind of testing? And this is just even from the simplest testing, the immunoperoxidase staining. If you get beyond staining for AL by immunofluorescents, etc., and AA, how many stain for transthyretin? If you went around the country, Steve, do you think most centers are staining for this or are even capable? Do they have the immuno tag?

Steve Bonsib: I don’t really know what that answer would be. We certainly do at Indiana. I would hope since that is the third most common type of amyloid and we have a reliable immunostain, most labs would have it. Certainly the other familial forms—I mean, those issues will come up so infrequently that our antibodies would outdate, and probably most people would not be able to go very far down that differential.

Gerald Appel: I think that’s Mel Schwartz in the second row there. Mel, do you do staining routinely, or can you stain for transthyretin, or would you have to send this out to somebody else?

Mel Schwartz: I’d have to send it to Steve Bonsib.

Gerald Appel: Dr. Mel Schwartz from a major clinical renal center would have to send it out to Steve, and I would do the same at Columbia University.

Maria Pickin: May I make a comment here? I’m Maria Pickin from Chicago. First of all, I would like to congratulate on the selection of the case. It’s about time that we, as a community, both renal pathologists and nephrologists, recognize that amyloid is not just one disease. Amyloid is not just AL, and then maybe it’s once in a while AA, but there are more different entities. And yes, it does matter because treatments are markedly different. Bone marrow transplant, we heard about this earlier today. Liver transplant. There are also new therapies on the horizon. We have seen the study on Fibrilex, etc., so it does matter a lot, and we need to make the diagnosis with confidence.

I would recommend that we all take a look at the early Summer issue of Blood, where Ray Comenza from Sloane-Kettering published a series which was designed in a similar fashion as that of Helen Lachmann, published in 2002 in New England Journal of Medicine. Similar study, no family history of amyloidosis, yet patients were diagnosed with familial amyloidosis. And this fact is also extremely important that people talk about variable gene penetrants, and we should not expect to get the family history of amyloidosis in the patient who is ultimately diagnosed with familial amyloidosis.

Now the issue is really very critical that the diagnosis is being made with confidence, and it has to be based on the amyloid protein deposited within the affected tissue. There are studies now coming out where people were diagnosed with abnormalities with amino acid substitutions in some of the proteins, which have been shown to be associated with amyloid, but that in itself does not necessarily prove that the amyloid, which is found in their organs, is of the hereditary type. So we need to be really extremely precise what we diagnose.

Gerald Appel: But we may need a national center as they have in London. Yes, they can all get the testing in London because they all send it to the same place, but we’re at Columbia University, and we’re just like Mel is in Chicago. We have to send it somewhere.

Maria Pickin: Well, I do routinely, my amyloid panel, I do stain against a panel of antibodies. I think the issue of guidelines for amyloid typing is long overdue and was addressed at the International Amyloidosis Conference. I do test routinely for the amyloid P component, which is a built-in control, present in all types of amyloids, so I have a reference. I stain for lambda light chain for AA, and, yes, at least one each of two of the most common hereditary types, transthyretin and fibrinogen. This can be done with greater confidence, in my view, and frozen sections using immunofluorescents, but it also can be done in paraffin sections, using immunoperoxidase.

Gerald Appel: Well, thank you for those comments. So we have at least two centers that can do this, and the rest of us may have to localize a center to do this. What should we do here, Steve? Having looked at this, what should we do about this lady’s family? Should we be testing them?

Steve Korbet: Yes, I would think if she has any family members, they should be watched very closely.

Gerald Appel: Should we be snipping a skin biopsy or looking at their gut?

Steve Korbet: Well, see whether they have any evidence of neuropathy, and then they should be worked up further.

Maria Pickin If I may add to this, I think the issue of the variable gene penetrants has to be stressed, and the presence of mutation does not necessarily mean that the people will develop the disease or at which point in time they will develop it. Steve has shown the hot spots for ATTR, which is amyloid derived from transthyretin, and, for example, the same mutation among the Portuguese patient will have a different clinical phenotype when compared with the mutation present in Swedish patients. So there are these, you know, environmental modifiers, etc., so I think there is a lot more to come.

Gerald Appel.

So we’ll take a careful history about that.

Helen Lachmann: Hi, Helen Lachmann from London. Thank
you very much, I thought that was wonderful, and not just for the obvious reasons. I wanted to make a couple of comments and then ask a question. In TTR amyloid at this stage, liver transplantation has no role. This woman would just die on the table. And even with a combined heart transplantation, she might very well do badly and with advanced neuropathy would not be accepted. As a result, genetic sequencing, and particularly TTR-amyloid in unaffected family members, is a very, very difficult ethical issue, because not all of these mutations will be penetrant, and not all of them are treatable, and particularly individuals under 30, we’re burdening them with a huge potential risk and, at present, for many of them, no effective treatment, and, therefore, my view is that most of these patients should be told of the possibility but should only be offered genetic sequencing after very skilled counseling.

My other two comments were I thought that was wonderful. The \( \lambda \) light chains is mean. In our series, we did pick up monoclonal hemopathies in 25% of patients, but they were almost all of them low-grade, incidental, IgG \( \kappa \), which is the commonest of monoclonal gammopathies of uncertain significance. Free \( \lambda \) light chains is a much more significant finding in amyloid and would push many people down the diagnosis of AL without thinking of a genetic disease. And finally, does anybody know what the mutation was?

Gerald Appel: No, I don’t have that information.

Helen Lachmann: Oh. Just for curiosity. Thank you.

Gerald Appel: Thank you very much for those comments. You know, that’s one other issue about the free \( \lambda \) light chains. Very commonly, you get patients for consultation sent in with a small amount of free \( \lambda \) light chain or free \( \kappa \). For whatever reason, we’re getting these sent in all of the time, and I never know what to make of them. Steve, do you see the same thing?

Steve Korbet: We’ve talked about this issue frequently at our institution because we’ll see someone for the evaluation of proteinuria, and as part of the workup, especially in an older person, we’ll do an immunofixation of urine and serum. We would do a renal biopsy in most of these patients anyway, but once we identify the presence of a paraprotein, our expectation is that the renal lesion may be associated. I think that’s where Dr. Agnes Fogo’s paper was very helpful by showing that over 60% of patients with paraproteins had an unrelated lesion on renal biopsy (4). I think now with the availability of even more sensitive assays, more patients will be identified with low levels of paraproteins. The important point is that oftentimes, paraprotein-associated diseases are often diagnosed by renal biopsy in the evaluation of proteinuria, and the sooner a diagnosis can be established, the sooner you can initiate appropriate treatment and hopefully have a positive impact. Once the disease has progressed too far (i.e., advanced and irreversible nephropathy, neuropathy, or cardiomyopathy), the likelihood that treatment, such as a liver transplant in FAP, will be helpful is minimal.

Gerald Appel: I thank you, Steve, that was an amazing presentation. My question is more banal. You didn’t comment on the hypertension of these patients. I had thought that amyloid patients were all hypertensive. Is this characteristic of these patients?

Steve Korbet: We usually think of them as having orthostatic hypotension. When they have advanced renal disease, they can actually have hypertension. Hypertension is seen about 30% of the time.

Gerald Appel: You know, one thing that amazes me is that I’m not sure how many people in the audience would have done the biopsy. This is a 68-year-old lady who has hypertension, 1.2 g of proteinuria, and a creatinine that’s already 3.8, and somebody could easily have said, if she didn’t have the family history, “This is a lady who has hypertension and progressive disease. What am I going to do differently if I get a biopsy?” Would everybody in the audience have done the biopsy? How many people would have done the biopsy? Liars! How many people—Dr. Lewis, would you have done the biopsy? Ed, would you have done the biopsy in this patient? Okay, so the answer is yes, they would have done it, so concurrent with most of you there.

Maria Pickin: Yes, I would like to add what Helen just said regarding the treatment of amyloid. Things are moving forward and things are developing. Liver transplantation was first performed over a decade ago in Sweden, and it is now known that in order for it to be useful, it has to be performed early on. So, yes, in the case of this patient, probably liver transplantation is out of the question. So we want to diagnose the patients as early as possible, at the very early stage of clinically significant amyloidosis. Now, the issue of testing is a difficult one, it’s a tough one, and I do agree with what Helen said. It has been shown that before clinical evidence of a disease, amyloid deposition, affected patients have deposits of the protein itself within the nerves. Researchers in Portugal have done several studies, and she documented that.

I also wanted to indicate that there are some alternative treatments which are on the horizon, pertaining to treatment of ATTR, transthyretin-derived amyloidosis, and there are some small molecular compounds. One of them is diflunisal, and Boston University has now opened a trial, offering treatment with diflunisal, which is an NSAID-type compound, which is supposed to stabilize affected transthyretin protein. It’s a tetramer, and if you have amino acid substitution, then the tetramer is no longer stable and you have these monomers floating around, and it has been shown that the monomers are actually detrimental, not tetramers. So things are moving forward, things are developing, and we need to be more careful with diagnosing amyloidosis and doing it with confidence.

Gerald Appel: Yes, and if there are people who have specific questions, since we have some experts right here to answer questions or get in touch with. I want to thank both Steve Bonsib for excellent pathology presentation and working this case up to the hilt. I also want to thank Steve Korbet, not just for getting the right answer, which I still think is impressive, but the fact that he did such a good job in presenting and worked through a great differential down to exactly what was the correct answer here. So I think you both did a terrific job. You’re not fired, Steve. You come back next year to do it again!

Disclosure

None.
References