

Nanobacteria-caused Mitral Valve Calciphylaxis in a Man with Diabetic Renal Failure

Tomislav M. Jelic, MD, PHD, Amer M. Malas, MD, Samuel S. Groves, MD, Bo Jin, MD, Paul F. Mellen, MD, Garry Osborne, MT, Rod Roque, MT, James G. Rosencrance, MD, and Ho-Huang Chang, MD

Abstract: We have found that nanobacteria, recently discovered Gram-negative atypical bacteria, can cause local calciphylaxis on the mitral valve in a setting of high-calcium X phosphorous product in the blood. We present the case of a 33-year-old man with diabetic renal failure on continuous ambulatory peritoneal dialysis who died as a result of multiple brain infarcts due to embolizations from mitral valve vegetations. Systemic calciphylaxis was not present. Spectrometric analysis of the mitral valve vegetations showed that they were composed of calcium phosphate, carbonate apatite form, and fibrin. The electron microscopy of the thrombotic vegetation demonstrated nanobacterium as a nidus for carbonate apatite formation. Investigation for the presence of nanobacteria in the multiple organs involved in systemic calciphylaxis may be of help in elucidating the pathogenesis of this frequently fatal disorder.

Calciphylaxis is a rare, often fatal complication of the end stage renal disease¹ characterized by systemic deposition of calcium phosphate salts (calcification) in the medial layer of the arteries, and in the soft tissues. Calcification of the media is followed by fibrous hyperplasia of the intima with obliteration of the arterial lumen and tissue gangrene.² Nanobacteria are the smallest (100–500 nm) recently discovered bacteria³ that produce carbonate apatite on their cell wall envelopes, and may be associated with stone formation and calcium deposition.⁴ We report a case of nanobacteria associated mitral valve calciphylaxis in a uremic diabetic patient.

Case Report

Patient's history, physical status, clinical-laboratory evaluations and autopsy findings were reviewed and correlated. Spectrometric analysis of the mitral valve calcifications was performed by the standard protocol in the Oncosite Laboratory of Dianon Systems, Stratford, CT. Dissected mitral valve vegetation tissue fixed in formalin was processed for electron microscopy and stained with uranyl acetate. Transmission electron microscopy was performed in our hospital on Zeiss model CEM 902 instrument manufactured in West Germany. Hematoxylin and eosin stained sections of the paraffin embedded blocks were performed according to standard protocols.

A 33-year-old man, father of two children was transferred for a second opinion on hand ischemia. The patient had a six week history of painful, dry scaling skin on the distal portion of the 3rd and 4th fingers of both hands which progressed to black discoloration and dry gangrene associated with marked pain. His past medical history was significant for type 1 diabetes mellitus for 30 years, diabetic nephropathy with end stage kidney disease requiring peritoneal dialysis for three years, and arterial hypertension. History was not suggestive of connective tissue disease or autoimmune disorder. Patient did use smokeless tobacco for approximately 15 years.

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Key Points

- Nanobacteria can cause local calciphylaxis on the mitral valve in a setting of high calcium X phosphorous product in the blood.
- Nanobacteria can cause disease in humans outside the urinary tract.
- Investigation for the presence of nanobacteria in systemic calciphylaxis may be of help in elucidating the pathogenesis of this frequently fatal disease.

From the Department of Pathology, Charleston Area Medical Center, and the Department of Internal Medicine, Robert C. Byrd Health Science Center, West Virginia University, Charleston Division, Charleston, WV, and the Department of Pathology, Ball Memorial Hospital, Muncie, IN.

Reprint requests to Tomislav M. Jelic, MD, PhD, Department of Pathology, Memorial Hospital, Charleston Area Medical Center, 3200 MacCorkle Avenue SE, Charleston, WV 25304. Email: tomislav.jelic@camc.org

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The initial examination revealed an afebrile patient with stable vital signs. The heart had regular rate and rhythm and without murmur. Lungs were clear and abdomen was benign. Neurologic examination revealed normal strength and reflexes throughout. Dry gangrene was noted on the 3rd and 4th fingers bilaterally but palpable radial and ulnar pulses were present. No evidence of ischemia was noted on the feet.

Additional blood studies obtained during the patient's hospitalization were as follows: Na 124 mEq/L, K 3.7 mEq/L, CO₂ 19 mm Hg, BUN 52 mg/dl, creatinine 11.3 mg/dl, glucose 69 mg/dl, calcium 10.1 mg/dl, phosphorus 9.9 mg/dl, erythrocyte sedimentation rate 128/h, white blood count 12.4, hemoglobin 9.5 mg/dl, hematocrit 28.9%, platelet count 520 K, PT 11 seconds (normal, 9.4–12.5 s), PTT 49.1 second (normal, 23.2–34.2 s), and INR, 1.0. Parathyroid hormone was within normal limits 31.5 pg/μl (normal 12–72 pg/μl). Cryoglobulin, *p*-antineutrophilic cytoplasmic antibody, *c*-antineutrophilic cytoplasmic antibody, antinuclear antibody, rheumatoid factor, and anti-thrombin III, were all negative. Protein C was 56% (normal, 66–129%), protein S 104.9 (normal, 62–145%). Factor V was 81 (normal, 50–150). Lupus anticoagulant was weakly positive, anti-double-stranded DNA was <10 (normal, <10), and cardiolipin antibody was negative.

A vascular surgeon evaluated the patient and performed a muscle biopsy which showed no evidence of vasculitis or any other abnormalities in the muscle or connective tissue. The patient was initially treated with IV heparin and steroids with dramatic clinical improvement. However, he suddenly developed difficulty swallowing and magnetic resonance image of the brain showed multiple small infarcts consistent with embolic disease. Cardiac echocardiogram revealed mitral valve vegetations. Transesophageal echocardiogram confirmed a presence of mitral valve vegetations and showed no mitral valve annular calcifications. Blood cultures were drawn and the patient was started on IV vancomycin, and gentamicin. He subsequently developed a painful, mottled, and pulseless right foot. Computerized tomography of the abdomen revealed a

splenic infarct. Patient's level of consciousness decreased and he required mechanical ventilation. After several days with no improvement, the patient's family desired withdrawal of ventilatory support and the patient expired.

Autopsy findings included mitral valve vegetations, brain emboli, bronchopneumonia, splenic infarct, and gangrene of the hand and foot. The vegetations on the mitral valve involved both anterior and posterior leaflets. They varied in size from 0.3 to 1.2 × 0.8 × 0.7 cm. One of the vegetations was polypoid in appearance and it measured 1.3 × 0.3 × 0.2 cm. All vegetations were gritty on cut sections and some of them were firm and stone-like. Vegetations attached to the mitral valve (Fig. 1) were composed of fibrin network containing numerous precipitates of calcium. Inflammatory cells were absent in the vegetations or mitral valve tissue. Crystallographic analysis (Fig. 2) of one of the vegetations, which looked like a kidney stone, showed it to be composed of calcium phosphate, carbonate form (carbonate apatite). Multiple infarcts caused by fibrin emboli were present in the brain. Sections of the kidneys demonstrated end stage kidney disease due to the advanced diabetic nephropathy (Kimmelstiel-Wilson syndrome). Standard postmortem cultures from the mitral valve vegetations and from the spleen were negative. Acute bronchopneumonia was present in both lower lung lobes and in the right middle lobe. *Enterobacter aerogenes* was cultured from the induced sputum. Transmission electron microscopy of the mitral valve vegetations showed numerous intracellular calcifications (Fig. 3). The nidus for their formation was nanobacterium. Electron-lucent nanobacterium is surrounded with electron dense shell of carbonate apatite (Fig. 4). Multiple electron dense nanobacteria are demonstrated on Figure 5. "Budding" of the nanobacteria is also depicted in Figure 5. Nanobacteria tend to form clusters (Fig. 5 and 6). Further buildup of carbonate apatite around clusters of nanobacteria entraps them in the electron dense aggregates with "hairy" appearance (Fig. 6). Size of nanobacteria in our patient varies from 42 to 300 nm (0.042–0.3 μm). There were no vasculitis or atherosclerotic changes in the gangrenous extremities and no coronary atherosclerosis.

Discussion

Nanobacteria are the smallest (100–500 nm) cultivatable replicating agents on earth, recently discovered in bovine and human blood by Kajander et al.³ Nanobacteria are cytotoxic, filtrable, Gram-negative, atypical bacteria. Their clinical relevance is unknown and in a phase of investigation. Some authors even doubt the mere existence of nanobacteria.⁵ Nanobacteria characteristically produce carbonate apatite on their cell enve-

lope⁴ and have been implicated in the pathogenesis of intracellular and extracellular calcification,⁴ kidney stone formation,⁶ and polycystic kidney disease.⁷

Calciophylaxis is a rare, often fatal, systemic disorder characterized by deposition (precipitation) of calcium phosphate salts (calcification) in the medial layer of the arteries and soft tissues.^{1,8,9} Calcification of the media is followed by fibrous hyperplasia of the intima with obliteration of the lumen and

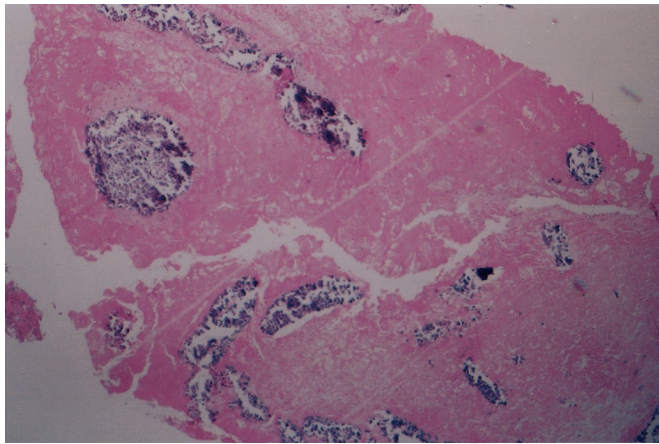


Fig. 1 Mitral valve vegetation composed of fibrin thrombus with calcifications. (Hematoxylin and eosin stain; original magnification, $\times 40$.)

tissue ischemia, necrosis, and gangrene.^{2,10} The first description of calciphylaxis was published in 1962.¹¹ Fewer than 200 cases have been reported so far.

Localized calciphylaxis of the mitral valve with formation of vegetations and subsequent thromboembolic disease in a patient with end stage renal disease as well as nanobacteria causing disease in humans outside the urinary tract have

not, to our knowledge, been reported previously. Calcific cerebral embolism in systemic calciphylaxis from mitral annular calcification has been reported.¹²

Calciphylaxis occurs in patients with end stage renal disease. Almost all reported patients were on dialysis, or had recently received a renal transplant. A few cases had been described in predialysis end stage renal disease patients.^{1,8,9,13,14}

The pathogenesis of calciphylaxis is poorly understood.¹ Several risk factors have been reported as follows: end-stage renal disease, hypercalcemic states, abnormalities in calcium-phosphorus concentration, hypercoagulable states, morbid obesity and recent weight loss, impaired protein-C activity, use of calcium carbonate, recent use of prednisone and hyperparathyroidism. Mathematical formulation was suggested to help identify which of uremic patients will develop ischemic tissue necrosis.¹⁵ All of the mentioned risk factors are frequently present in end stage renal disease patients and it is not clear why calciphylaxis is so rare.

Calciphylaxis is known to occur in the hands, fingers and lower extremities mimicking atherosclerotic peripheral vascular disease. This patient presented with digital ischemia, followed later with splenic infarction and multiple ischemic brain infarcts. Postmortem examination did not demonstrate any of the characteristic findings of systemic calciphylaxis, although product of calcium concentration X phosphorus con-

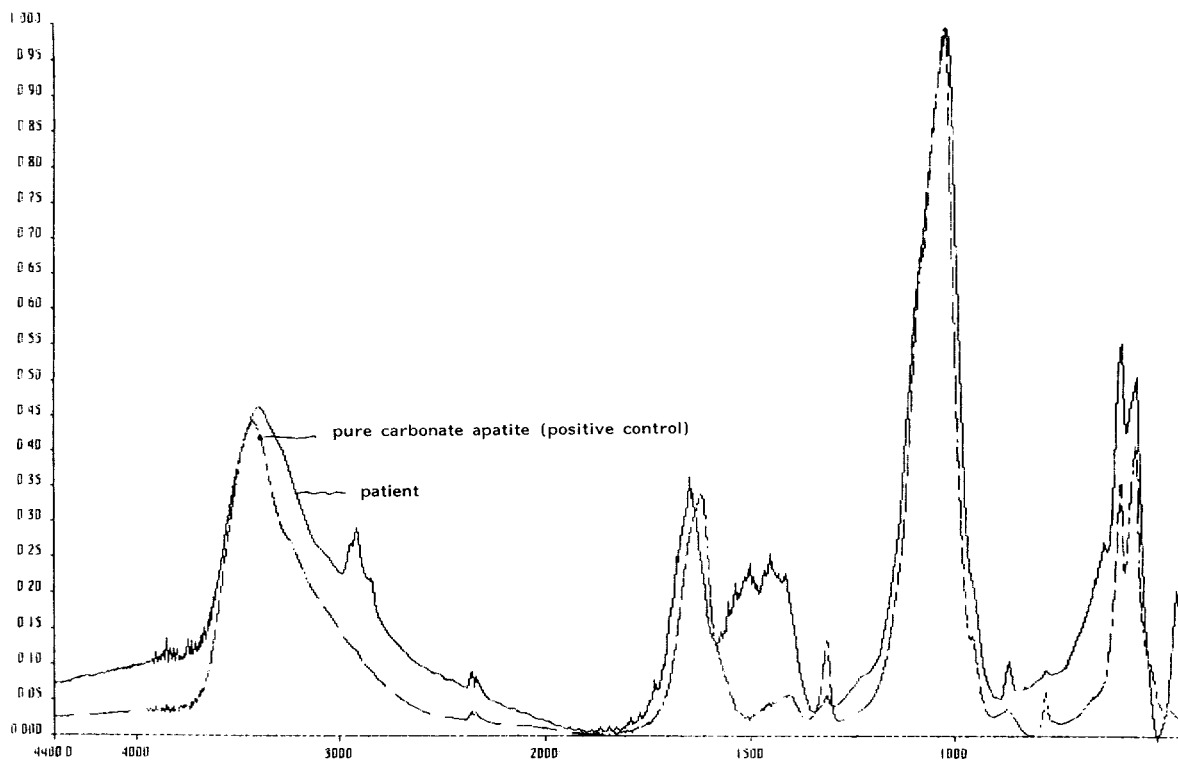


Fig. 2 Crystallographic analysis of the mitral valve calcifications showing calcium phosphate (carbonate apatite). Full line is patient's curve. Interrupted line is positive control.

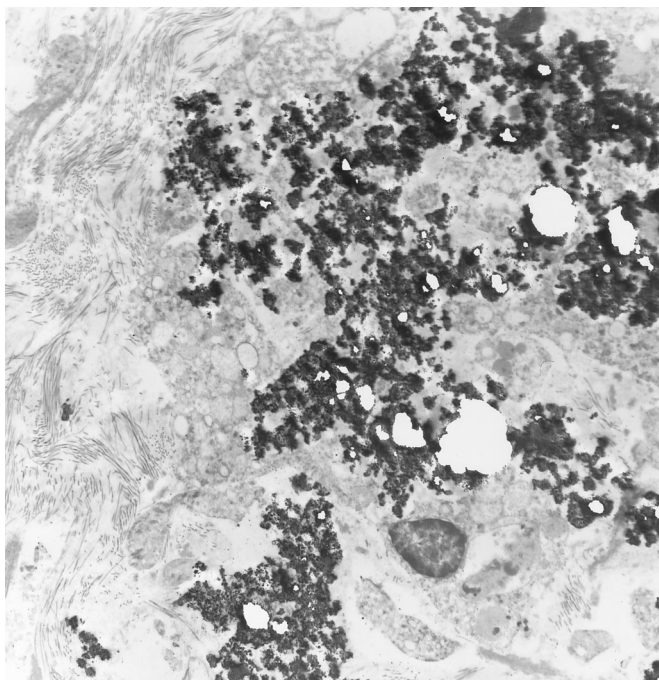


Fig. 3 Transmission electron microphotograph of the mitral valve vegetation showing numerous intracellular calcifications. (Original magnification, $\times 3,000$.)

centration in the blood in our patient was 100. The risk for systemic calciphylaxis is considerable when the calcium X phosphorus product exceeds 70.¹⁶

An autopsy revealed calcified thrombotic vegetations on the mitral valve and thrombotic emboli in the small arteries of



Fig. 4 Nanobacterium surrounded with the electron-dense shell of carbonate apatite. (Original magnification, $\times 50,000$.)

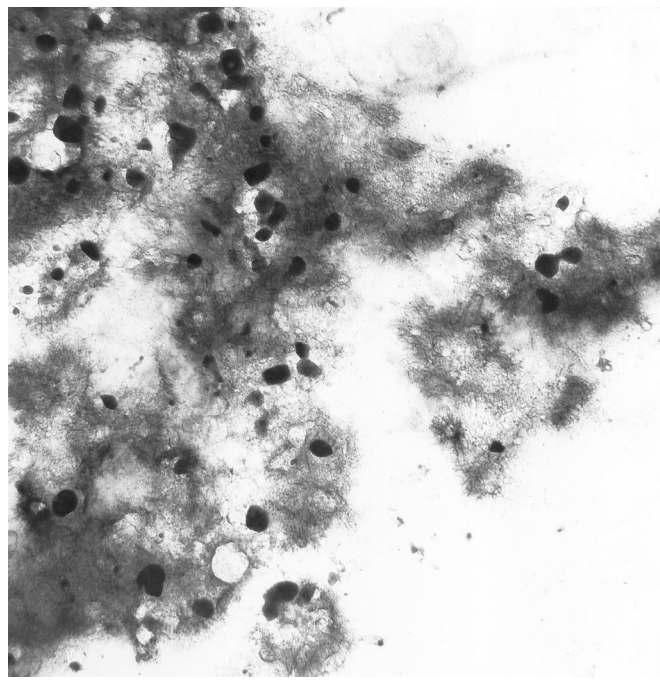


Fig. 5 Multiple electron-dense nanobacteria. Nanobacterium at the 3 o'clock position is budding. (Original magnification, $\times 50,000$.)

the brain. Thrombotic vegetations were source of emboli causing ischemic infarcts in the brain and spleen, gangrene of fingers and sudden ischemic changes in the left foot.

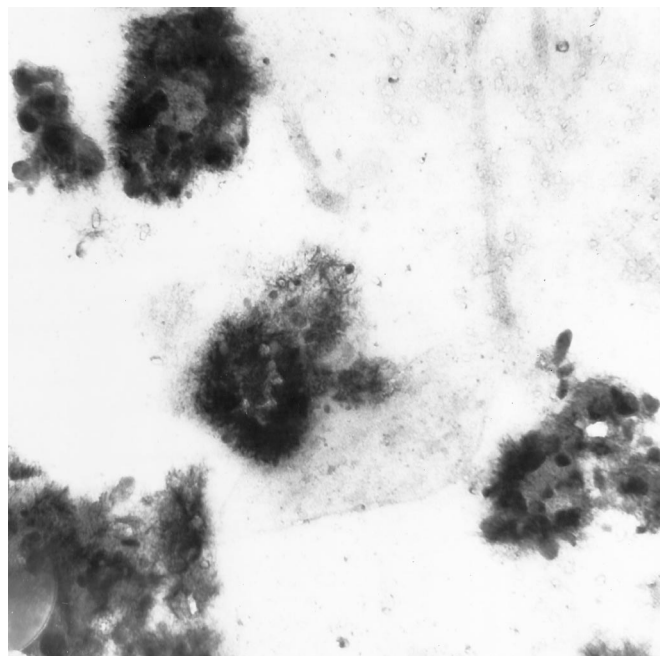


Fig. 6 “Hairy” appearance of nanobacteria due to the buildup of carbonate apatite envelope. (Original magnification, $\times 50,000$.)

Electron microscopy demonstrated nanobacterium as a nidus in the smallest calcium phosphate (carbonate apatite) units in the mitral valve vegetations.

This indicates that the synergistic action of nanobacteria colonized on the mitral valve and high calcium X phosphate product may have caused precipitation of calcium phosphate salts on mitral valve. Thrombotic build up of fibrin followed, likely caused by inactivation action of calcium phosphate on antithrombin III.¹⁷ Nanobacteria had not caused inflammatory reaction in the mitral valve. Our finding of nanobacteria at the spot where local calcification has occurred in a patient with predisposition for systemic calciphylaxis (which itself was not present) indicates that nanobacteria are possibly an essential factor in developing local calciphylaxis. We think that rarity of the calciphylaxis in the patients with chronic renal failure (end-stage kidney disease) and high calcium X phosphorus product is due to the absence of nanobacteria. Presence of nanobacteria in the various organs involved in systemic calciphylaxis has not been investigated. Our finding suggests that it may be of help to investigate for presence of nanobacteria in systemic calciphylaxis.

Conclusion

Nanobacteria in the mitral valve can cause calcium phosphate calcifications (local calciphylaxis) in the form of carbonate apatite in a patient on continuous ambulatory peritoneal dialysis who has increased blood calcium concentration and phosphorus concentration product. Investigation for the presence of nanobacteria in the multiple organs involved in systemic calciphylaxis may be of help in elucidating of the pathogenesis of this frequently fatal process.

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