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Granulomatous inflammation and hypercalcemia in patients with severe systemic oxalosis

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Key words

Systemic oxalosis, primary hyperoxaluria, hypercalcemia, inflammation, granuloma, bone, fractures, FDG-PET/CT, receptor activator of nuclear factor- κ B-ligand, CD68

Introduction

Primary hyperoxalurias (PHs) are rare genetic disorders that cause hyperproduction of oxalate in the liver. The clinical manifestations of excess oxalate include urolithiasis, nephrocalcinosis, and end-stage renal disease (ESRD). The onset of renal failure – which typically represents a turning point in the course of PHs – results in the inability to remove oxalate in the urine. This in turn leads to a progressive accumulation of insoluble calcium oxalate (Ca-Ox) crystals in various tissues. This severe condition, termed systemic oxalosis (SO),¹ has an unfavorable prognosis characterized by persistent pain, increased vulnerability to fractures,² and significant mortality. SO is currently thought to result mainly from diagnostic delays or limited access to optimal care. A clinically feasible way to reduce the risk of SO in patients with PHs is simultaneous liver-kidney transplantation (SLKT) performed at an early disease stage. A novel therapeutic approach lies in the use of RNA interference therapy – which may be especially well suited at reducing the hepatic hyperproduction of oxalate.³

Data from animal models have suggested that inflammation may play a role in the pathogenesis of oxalosis.⁴ Additionally, bone accumulation of Ca-Ox crystals in patients with SO has been shown to elicit the formation of granulomas² – whose presence may be related to the onset of hypercalcemia.⁵ Notwithstanding these preliminary observations, the clinical impact of inflammation and hypercalcemia during the course of SO has not been fully elucidated. This multicenter case series was aimed at investigating this issue in five adult patients with PH complicated by ESRD. ¹⁸F-fluorodeoxyglucose positron-emission tomography/computed tomography (FDG-PET/CT) data as well as histology and laboratory findings were obtained in the context of clinical care and retrospectively reviewed and analyzed in relation to clinical outcomes.

Case presentation

The five study patients were 3 men and 2 women recruited from four French Nephrology centers. The median age at the beginning of dialysis was 34 years (range: 25–62 years). The diagnosis of PH type 1 and PH type 2 was confirmed by mutation analysis of the *AGXT* gene in patients #1, #2, #4, and #5 and of the *GRHP* gene in patient #3, respectively. Three of the five study patients were diagnosed with PH after the initiation of dialysis. The median duration of dialysis before LT/SLKT was 7 years (range: 4–9 years). Patient follow-up was concluded in November 2020. The median follow-up time after transplantation was 24 months (range: 16–54 months). The general characteristics, laboratory findings, and clinical outcomes of the study patients are summarized in **Table 1**.

Imaging findings

The initial FDG-PET/CT scans were performed 4 and 10 months before LT/SLKT and 1, 12, and 15 months after transplantation in patients #2, 4, 5, 1, and 3, respectively (**Figures 1 and S1**). All participants showed diffuse joint, bone, and soft tissue hypermetabolic lesions in areas of calcium depositions. Osseous lesions of the spine either involved the vertebral body (with evidence of spondylitis in patients #2, 3, and 4) or the posterior elements (leading to spinal cord compression in patient #1). A biopsy of a hypermetabolic vertebral lesion in patient #1 revealed the presence of an inflammatory granulomatous reaction elicited by Ca-Ox crystals (**Figure S3, panels K-O**). CT images obtained using the “bone window” settings identified the presence of reactive bone resorption areas adjacent to the Ca-Ox deposits, which were associated with fractures. A longitudinal scan performed in patient #2 revealed a rapid progression of valvular and vascular calcifications (**figure S1**). While repeated FDG-PET/CT examinations after SLKT showed that hypermetabolic lesions tended to persist over time (as long as four post-transplant years in patient #1), FDG avidity of the foci decreased significantly. Bone scintigraphy in

patients #1 and 4 revealed mild tracer uptake in bone areas surrounding calcium deposition, which corresponded to the bone resorptive lesions (**Table S2**).

Laboratory findings

Hypoalbuminemia and hypercalcemia were observed in all participants (**Tables 1 and Table S1**). While the presence of hypercalcemia was reflected by high serum ionized calcium or corrected calcium levels, total serum calcium was generally within the reference range. Patients #1 and 2 developed hypercalcemia after 3 and 4 years on dialysis, respectively; the event was concomitant to the onset of bone pain and persisted at follow-up. A complete diagnostic workup ruled out the presence of malignancies. Because of hypercalcemia and ESRD, hyperparathyroidism was frequently suspected. However, specific drug treatments were ineffective at lowering hypercalcemia – suggesting that it was unrelated to PTH. Increased ionized calcium was accompanied by elevated serum biomarkers of bone turnover.

In addition, biomarkers of chronic granulomatous disease – including serum levels of ACE and/or 1,25OH₂D – were elevated in all participants. Patients #2, 3, and 4 showed laboratory signs of a chronic inflammatory syndrome. Serum levels of IL-6, IL-1, and TNF- α were quantified in three cases and IL-6 was consistently increased. Elevated serum oxalate levels did not generally regress in the post-transplantation period and – in patient #1 – they were still evident at four years after SKLT. Patients #1 and 2 showed increased urinary oxalate and calcium concentrations coupled with crystalluria (whewellite and/or weddellite).

Histology results

Within the context of clinical care, patients #1, 2, 4, and 5 underwent biopsies of hypermetabolic lesions located in different tissues (bone and soft tissues; **Table S2**). Histology examination revealed the presence of reactive granulomas consisting of both multinucleate

giant cells (MGC) and macrophages that typically surrounded Ca-Ox crystals (**Figure S2**).

MGC in contact with the trabecular surfaces exhibited an osteoclast-like activity, which led to bone resorption. Immunohistochemistry of bone and nodel biopsies (patient #1) as well as of hypermetabolic calcified muscle lesions (patient #2) revealed that MGC abundantly expressed CD68 and RANK-L (**Figure S3**).

Clinical course (Table 1)

All participants showed recurrence of crystal nephropathy in the kidney graft. Patient #1 had obstructive calculi on the kidney graft. Recurrence of nephropathy lead to early renal failure after the first kidney transplantation in patients #4 and in patient #3 (who had a diagnosis of type 2 PH)⁶. At the end of the follow-up, two patients lost their kidney graft and the remaining three had severe renal dysfunction. Diffuse valvular calcifications – which were likely related to hypercalcemia – required replacement valve surgery in patients #1 and 2. All cases had a history of venous thrombosis and cutaneous necrosis occurred in three patients. Four patients had fractures and rheumatic pain. Pain slowly regressed after transplantation in parallel with the reduction of FDG avidity in hypermetabolic foci on FDG-PET/CT. The clinical outcomes were generally unfavorable. Patients # 2, 3, and 4 died of sepsis and/or cachexia at the age of 35, 43, and 40 years, respectively.

Treatment approach of hypercalcemia, fractures, and crystal rheumatism

While patients #1, 2, 3, and 4 received bone antiresorptive agents (bisphosphonates and/or denosumab) for hypercalcemia and fractures after SLKT, their efficacy in normalizing calcium levels was transient (**Table 1**). Patients #1, 2, and 3 were treated with subcutaneous injections of denosumab – a monoclonal antibody against RANK-L; this approach effectively but temporarily reduced serum calcium and bone turnover biomarkers. Consequently, a prolonged

treatment was necessary. Patients #1, 2, and 3 received high-dose corticosteroids during LT/SLKT induction and/or because of acute graft rejection. While steroids induced a temporary remission of hypercalcemia, relapses were evident when these drugs were tapered off. Patient #2 received solumedrol boluses at induction – which led to a dramatic reduction of crystal arthropathy-associated pain and inflammation. Unfortunately, the disabling pain relapsed after a few months. A further therapeutic attempt with infliximab was unsuccessful, whereas the IL-1 receptor antagonist anakinra was rapidly withdrawn owing to infectious complications of cutaneous tophi.

Discussion

Supplement material: discussion.

In conclusion, our data illustrate for the first time the key role played by reactive granulomatous inflammation as a key driver for the high morbidity (i.e., crystal arthropathy, fractures, recurrent crystal nephropathy, and cardiovascular complications) and mortality observed in patients with severe SO. FDG-PET/CT and biological markers may help clinicians to assess the severity of oxalosis and the risk of recurrent crystal nephropathy after kidney transplantation (**Table 2**).

Disclosures

Professor Bruno Moulin serves as an advisory board member and received research grants from Alnylam Pharmaceuticals. All of the other authors declare no conflicts of interest in relation to this manuscript.

Patient consent

This is a retrospective review of prospectively collected data in the context of clinical care. According to current French regulations, the need for institutional review board approval was waived due to the study design. Written informed consent for additional analyses of biopsies (immunohistochemistry) for research purposes was obtained from patients #1 and #2; these analyses were approved by the local Institutional Review Board (approval number: DC-2013-1990).

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Main figure legend

Figure 1. Illustrative ^{18}F -fluorodeoxyglucose positron-emission tomography/computed tomography (FDG-PET/CT) images in the five study patients

FDG-PET/CT whole-body images for patients #1 (a, b), #2 (c, d), #3 (e, f), #4 (g, h), and #5 (i) revealed bilateral diffuse hypermetabolic lesions throughout the entire skeleton. Additionally, numerous joints were affected (shoulder girdle joints, chondrocostal and costovertebral joints, spine joints, pelvic girdle joints, and limb joints).

Patients #2 (c, d), #3 (e, f), and #4 (g, h) had evidence of spondylitis; we also identified the presence of sacroiliitis in patients #2 (c), #3 (e), and #4 (g). Foci of increased FDG uptake were identified at multiple muscle insertions, cutaneous areas, and cartilages (larynx). Patient #2 showed bulk hypermetabolic calcified muscular masses at the hip (c). The intensity and number of hypermetabolic lesions were lower for patient #5 (i).

Table 1. General characteristics, clinical outcomes, laboratory findings, and treatment approaches for hypercalcemia in the five study patients

| | Patient #1 | Patient #2 | Patient #3 | Patient #4 | Patient #5 |
|--|---|--|---|---|---------------------------|
| Sex | F | M | M | M | F |
| PH type | 1 | 1 | 2 | 1 | 1 |
| Age at diagnosis, years (circumstances of diagnosis) | 67 (spinal cord compression by Ca-Ox deposits) | 5 (nephrocalcinosis) | Teenager (urolithiasis) | 35 (kidney graft failure) | 51 (Pre-KT evaluation) |
| Renal presentation | Urolithiasis, ESRD | Nephrocalcinosis, urolithiasis, ESRD | Urolithiasis, ESRD | ESRD | Urolithiasis, ESRD |
| Age at the beginning of dialysis, years (calendar year) | 62, 2009 | 25, 2010 | 34, 2009 | 33, 2013 | 47, 2012 |
| Dialysis duration before SLKT/LT, years | 7 | 8 | 9 | 4 | 7 |
| Transplantation, type and date | SLKT in 2016 | SLKT in 2018 | KT in 2016, SLKT in 2018 | KT in 2015, LT in 2017 | SLKT in 2019 |
| Clinical outcomes | | | | | |
| Kidney graft outcome | | | | | |
| Early outcome | DGF | Primary dysfunction | Early failure of the first kidney graft due to recurrent crystal nephropathy | Early graft failure due to graft venous thrombosis | DGF |
| Graft crystals on biopsy | Yes | Yes | Yes | Yes | Yes |
| Graft lithiasis | Yes (obstructive lithiasis requiring long-term pyelostomia) | No | No | No | No |
| GFR (ml/min/1.73 m ²) at last follow-up | 15 | Hemodialysis | 28 | Hemodialysis | 23 |
| Bone outcomes | | | | | |
| Fractures (localization) | Yes (foot) | Yes (vertebra) | Yes (vertebra, humerus) | Yes (vertebra) | No |
| Rheumatologic pain | Pain (spine, extremities) that led to an impaired mobility; appearance three years after the beginning of dialysis Spinal cord compression | Intense pain that limited mobility (wheelchair) and that required treatment with morphine; appearance five years after the beginning of dialysis | Rheumatic pain | Chronic pain, knee crystalline arthritis, Achilles tendon rupture | No |
| Cardiovascular events | Calcific aortic stenosis requiring valve replacement, thrombosis of the fistula | Transient ischemic attack, calcific mitral stenosis requiring valve replacement, thrombosis of the fistula | Calcific mitralic stenosis with heart disease, thrombosis of the fistula | Thrombosis of the fistula, lower limb DVT, pulmonary embolism | Thrombosis of the fistula |
| Other clinical manifestations | - | Cutaneous necrosis, tophi on fingers, hepatosplenomegalia | Cutaneous necrosis | Pancytopenia | Cutaneous necrosis |
| Death (date, cause) | No | Yes (2020, calcific mitral stenosis, sepsis, cachexia, severe hypercalcemia) | Yes (2020, sepsis) | Yes (2020, sepsis, angiocholitis, cachexia) | No |
| Laboratory findings | | | | | |
| Hypercalcemia*, years of onset after the beginning of dialysis | Yes, 3 (2012) | Yes, 4 (2014) | Yes, 5 (2014) | Yes, 4 (2017) | Yes, NA |
| Hypoalbuminemia (< 30 g/L) | Yes | Yes | Yes | Yes | Yes |
| Chronic elevation of C-reactive protein | No | Yes | Yes | Yes | No |
| Angiotensin-converting enzyme | Elevated | Elevated | Upper limit of normal | Elevated | Elevated |
| 1,25 OH ₂ -vitaminD | Normal or transiently elevated | Elevated | Elevated | Elevated for a patient on dialysis | Low |
| Bone remodeling markers | Elevated | Elevated | Elevated | Elevated | Elevated |
| Treatment for hypercalcemia | Bisphosphonates, denosumab 60 mg every 2-3 months | Denosumab 60 mg and pamidronate | Bisphosphonates every 2 months followed by denosumab 60 mg every 3 months | Pamidronate in 2020 | No |
| Treatment for suspected hyperparathyroidism | Cinacalcet 30 mg/day after LKT | No | Parathyroidectomy in 2015 leading to hypoparathyroidism; no correction of hypercalcemia | Cinacalcet before LT | No |

Abbreviations: F, female; M, male; PH, primary hyperoxaluria; LT, liver transplantation; SLKT, simultaneous liver kidney transplantation; KT, kidney transplantation; BMD, bone mineral density; DVT, deep vein thrombosis, DGF, delayed graft function, ESRD, end-stage renal disease, NA, not available; Ca-Ox, calcium-oxalate *Hypercalcemia was defined by a serum ionized calcium > 1.3 mmol/L or a serum corrected calcium >2.6 mmol/L (> 10.4 mg/dL).

Table 2. Teaching points

1. Primary hyperoxaluria (PH) – a rare genetic disease characterized by the hepatic hyperproduction of oxalate – frequently leads to ESRD.
2. After renal failure, insoluble calcium oxalate crystals accumulate in various tissues – mainly in the bone – resulting in systemic oxalosis. Systemic oxalosis has unfavorable outcomes characterized by persistent pain, fractures, and significant mortality.
3. Early management of patients with PH and ESRD is paramount to avoid severe systemic oxalosis and recurrence of crystal nephropathy in the kidney allograft.
4. While current strategies to treat PH patients with ESRD mainly consist of dual liver–kidney transplantation, RNA interference therapy will likely lead to more tailored treatment options.
5. The role of inflammation in the pathogenesis of systemic oxalosis as well as in its clinical manifestations remains unclear.
6. In this case series of five patients with severe oxalosis, FDG-PET/CT images revealed diffuse joint, bone, and soft tissue hypermetabolic lesions, which corresponded to granulomas elicited by calcium-oxalate crystals. Hypermetabolic lesions – which were associated with bone resorption and fractures – did not regress after kidney transplantation. Laboratory findings revealed hypoalbuminemia and hypercalcemia accompanied by increased bone turnover and granulomatosis biomarkers. The clinical course was unfavorable and three patients died of disease. Collectively, these data illustrate the key role played by granulomatous inflammation as a driver of the high morbidity and mortality in patients with severe systemic oxalosis.
7. While bone antiresorptive agents and corticosteroids were effective in controlling hypercalcemia, they did not prevent recurrences.
8. FDG-PET/CT may serve as a promising imaging tool to assess the systemic burden of oxalosis.

Supplement materials

Figure legends

Figure S1. Illustrative ¹⁸F-fluorodeoxyglucose positron-emission tomography/computed tomography (FDG-PET/CT) images in patients #2 (a, b, g, h, i, j, m, n) and #3 (c, d, e, f)

Patterns of FDG uptake in patient #2: spinal images revealed the presence of spondylitis with a maximum standardized uptake value (SUVmax) of 14.6. Areas of increased tracer uptake were also noted in the sacroileal (SUVmax: 16.7), gluteal muscles (SUVmax: 7.5; a), sterno-clavicular joints (g), pelvis girdle (i), and hip-joint (h). Axial image showing hypermetabolic lesions in native kidneys (m). Patient #2 presented hepatosplenomegaly with slightly increased FDG uptake (a).

The corresponding CT images (“bone window” protocol) revealed that the hypermetabolic foci on PET were extensive calcium deposits (b, j, d, f); bone resorption was evident in the adjacent bone (e.g., endplate of the vertebrae [k] or cortical bones of the limbs [f]).

Patient #3 showed humeral hypermetabolic lesions (c, e) accompanied by bone erosion and thinning of the adjacent cortical area (d, f); these lesions resulted in a fracture of the right humerus (→; c, d).

Patient #3 showed severe aortic calcifications (k), whereas severe mitral valve calcifications were found in patient #1 (n).

Figure S2. Illustrative biopsy findings in three study patients

Panel A. Patient #4: iliac bone biopsy showing the presence of multinucleated giant cells (MGC) in proximity to Ca-Ox crystals (arranged in a star-like figure) adjacent to spongy bone trabeculae. When in contact to the trabecular surface, MGC acted in an osteoclast-like fashion and led to an increased percentage of the eroded surface. Osteoid tissue was observed between the crystals and the bone (hematoxylin-eosin-saffron staining; 400× magnification).

Panel B. Patient #1: iliac bone biopsy showing the presence of Ca-Ox crystals calcifications surrounded by a reactive inflammatory infiltrate that eroded the trabecular bone (→) (hematoxylin and eosin staining, 400× magnification).

Panel C. Patient #2: gluteal mass biopsy showing the presence of diffuse calcifications surrounded by a reactive inflammatory infiltrate with numerous MGC (hematoxylin and eosin staining, 400× magnification).

Figure S3. Immunohistochemical analysis of tissue inflammatory cells using the following markers: CD68 (column 2), CD163 (column 3), RANK (column 4), and RANK-L (column 5)

Panels A-E: Patient #2. On analyzing biopsies obtained from hypermetabolic calcified muscular masses, multinucleated giant cells surrounding Ca-Ox deposits were evident (A; hematoxylin and eosin staining, 400× magnification). Giant cells adjacent to calcifications were positive for CD68 (B; 200× magnification). While we found a weak RANK expression (D; 400× magnification), RANK-L was abundantly expressed (E; 400× magnification). CD163-positive cells were identified in the proximity of granulomatous lesions (C; 200× magnification).

F-J: Patient #1 showed a bone exostosis in the right hallux front. An osteoid matrix without osteoblasts but accompanied by an inflammatory infiltrate was identified adjacent to calcium-oxalate crystals (F; hematoxylin and eosin staining, 200× magnification). Immunochemical staining of multinucleated giant cells revealed that marker expression levels were as follows: high CD68 (G; 400× magnification), low CD163 (H; 400× magnification), low RANK (I; 400× magnification), and high RANK-L (J; 400× magnification).

K-O: L3–L4 vertebral bone biopsy obtained from patient #1 showing the presence of numerous Ca-Ox crystals surrounded by inflammatory cells (F; hematoxylin and eosin staining, 200× magnification). Immunochemical staining revealed that marker expression levels were as follows: high CD68 (K; 400× magnification), low CD163 (H; 400× magnification), low RANK (I; 400× magnification) and high RANK-L (J; 400× magnification).

P-T: Iliac bone biopsy obtained from patient #1 showing the presence of Ca-Ox crystals in bone marrow accompanied by a granulomatous reaction (K; hematoxylin and eosin staining, 400× magnification). Immunochemical staining of multinucleated giant cells that surrounded the Ca-Ox crystals revealed that marker expression levels were as follows: high CD68 (L; 400× magnification), high RANK-L (O; 400× magnification), low CD163 (R; 400× magnification), low RANK (N; 400× magnification).

U-Y: Lymph node biopsy obtained from patient #1 1: very large Ca-Ox crystal (U; hematoxylin and eosin staining, 200× magnification). Immunochemical staining of multinucleated giant cells revealed that marker expression levels were as follows: high CD68 (V; 400× magnification), low CD163 (W; 400× magnification), low RANK (X; 400× magnification) and high RANK-L (Y; 400× magnification).

The primary antibodies used for the detection of RANK and RANKL were obtained from LSBio (Seattle, WA, USA; catalogue number: LS-B11252 and LS-B1425, respectively).

Table S1. Laboratory findings in the five study patients

Table S2. Summary of imaging (FDG-PET/CT and bone scintigraphy) and histology findings in five patients with severe oxalosis

Supplement Discussion

