Published in final edited form as:

J Pediatr Hematol Oncol. 2008 August ; 30(8): 612–617. doi:10.1097/MPH.0b013e318168f072.

Hypocalcemia in a Patient With Osteosarcoma and 22q11.2 Deletion Syndrome

Francis J. Mussai, BM, BCh, MRCPCH^{*}, Lea C. Cunningham, MD^{*}, Geoffrey Rezvani, MD[†], Constantine A. Stratakis, MD, D(med)Sci[†], James C. Reynolds, MD[‡], Galina Nesterova, MD[§], Robert M. Henshaw, MD^{||}, Jason E. Levine, MD^{*}, Lee J. Helman, MD^{*}, Diane C. Arthur, MD[¶], and Su Young Kim, MD, PhD^{*}

^{*}Pediatric Oncology Branch, National Institutes of Health, Bethesda, MD

[¶]Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD

[†]Pediatric Endocrinology Training Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD

[‡]Nuclear Medicine Department, Clinical Center, National Institutes of Health, Bethesda, MD

[§]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

^{||}Department of Orthopedics, Washington Cancer Institute, Georgetown University School of Medicine, Washington, DC

Summary

Hypocalcemia is a rare complication of osteosarcoma, having been described in only 4 reports. We present the case of a 16-year-old male with metastatic osteosarcoma of the right humerus who was found to have severe asymptomatic hypocalcemia. Cytogenetic analysis of peripheral blood revealed a microdeletion in band 22q11.2. Following amputation of the tumor-bearing extremity, the patient's calcium levels increased, but did not normalize. These findings suggested that the etiology of his hypocalcemia was osteoblastic utilization of calcium by the tumor, exacerbated by 22q11.2 deletion syndrome.

Keywords

hypocalcemia; osteosarcoma; 22q11.2 deletion syndrome

Osteosarcoma is the most common malignant bone tumor in the United States. Advances in surgery and chemotherapy have resulted in cure rates that approach 70% for patients with localized disease.¹ However, patients with metastatic disease have only a 25% survival rate.

Hypocalcemia occurs in approximately 10% of patients with metastatic cancer.² The etiology remains unknown, and the theories range from ectopic production of a parathyroid hormone (PTH)-related peptide to the incorporation of calcium into the metastatic lesion. Only 4 cases of hypocalcemia and osteosarcoma have been reported.^{3–6} These patients

shared the common features of rapid normalization of calcium levels with supplementation, extensive metastatic disease, and early demise (Table 1).

We report the case of a 16-year-old male with a large primary osteosarcoma of the humerus with metastases to the lungs and the pelvis. He also had severe, refractory, asymptomatic hypocalcemia, despite supplementation with 29 g of calcium daily. This finding, in addition to the presence of a ventricular septal defect on echocardiogram, and his short stature, led us to explore other etiologies for the hypocalcemia. Cytogenetic analysis of peripheral blood revealed a 22q11.2 microdeletion.

Hemizygous microdeletion of 22q11.2 is associated with a heterogeneous group of disorders that includes DiGeorge syndrome, velocardiofacial syndrome, and others.⁷ Clinical phenotypes vary widely, and may include hypocalcemia, cardiac anomalies, thymic hypoplasia, cleft defects, short stature, behavioral problems, and others. Due to the wide range of both symptoms and syndromes, for patients who bear the deletion, the term 22q11.2 deletion syndrome is the most accurate description.

Following amputation of the tumor-bearing extremity, the patient's calcium level increased, but remained below normal. This finding suggested that the etiology of his hypocalcemia was osteoblastic utilization of calcium by the primary tumor, exacerbated by 22q11.2 deletion syndrome. We describe his case in more detail and discuss the possible connection between 22q11.2 deletion and osteosarcoma.

CASE REPORT

The patient is a 16-year-old male from Africa who presented with an 18-month history of right arm swelling. A biopsy was performed in his home country and he was given a diagnosis of chondrosarcoma and offered amputation. The patient and his family declined this option and they came to the United States 16 months later. X-rays and a computed tomographic scan revealed a large ($22 \times 19 \times 21$ cm) mass in the right humerus (Figs. 1A, B). Further workup for metastases revealed diffuse pulmonary involvement and 2 pelvic lesions (Fig. 1C). Biopsy of the primary mass resulted in a diagnosis of osteoblastic osteosarcoma.

His serum electrolytes were normal with the exception of a serum calcium of 1.75 mmol/L (nL 2.05 to 2.5) and an ionized calcium of 1.02 mmol/L (nL 1.22 to 1.38) (Fig. 2). Despite these low levels, the patient did not have paresthesia, tetany, Chvostek's sign, nor any other symptom associated with hypocalcemia. Urinary calcium concentrations were undetectable. Urine phosphorus excretion was not tested at the time. Other relevant laboratory values included the following: magnesium 0.72 mmol/L (nL 0.75 to 1), phosphorus 4.0 mg/dL (nL 2.5 to 4.8), blood urea nitrogen 3 mg/dL (nL 8 to 22), creatinine 0.4 mg/dL (nL 0.9 to 1.4), and albumin 1.9 g/dL (nL 3.7 to 4.7). Other values included an intact PTH level of 290 pg/mL (nL 16 to 87), a 25-hydroxy vitamin D level of 12 ng/mL (<10 is severely deficient), and a 1,25-dihydroxy vitamin D level of 45 pg/mL (nL 22 to 67).

A whole body dual energy x-ray absorptiometry scan gave a Z-score for bone mineral density of -0.9 (Fig. 3). However, 32% of his total body calcium was in the primary tumor. Subtraction of this area resulted in a corrected Z-score of -2.85 , a value that is extremely low for the patient's age. Correction for the patient's short stature (164 cm) by using grams of bone mineral content per cm height (replacing the right arm with a second left arm) also gave a low Z-score of $-2.36.8$ The patient was started on calcium supplementation, up to 29 g daily, in addition to calcitriol, up to 2.25 mcg/d. His ionized calcium improved marginally with this regimen, and his levels fluctuated between 0.92 and 1.15 mmol/L, but did not achieve normal steady state.

We believed that the patient's hypocalcemia was multi-factorial, including vitamin D deficiency, poor nutritional status, and most importantly, incorporation of calcium into the primary tumor and metastatic sites of disease. Although the patient was not obviously dysmorphic, the presence of a ventricular septal defect, short stature, and refractory hypocalcemia indicated testing for 22q11.2 microdeletion. High-resolution G-banding revealed a 46,XY male karyotype in 20 cells analyzed, with a microdeletion in band q11.2 of one chromosome 22 suspected in the prometaphase cells. Fluorescence in situ hybridization with the LSI TUPLE1 SpectrumOrange/LSI ARSA SpectrumGreen Probes (Abbott Molecular, Des Plaines, IL) showed that 1 copy of the TUPLE1 probe was deleted from band 22q11.2 in 15 of 15 metaphase spreads, and in 200 of 200 interphase nuclei, scored (Fig. 4A). There was no evidence of rearrangement or deletion of EWSR1 in 4 metaphase spreads, or 213 interphase nuclei, scored using the LSI EWSR1 (22q12) Dual Color Break Apart Rearrangement Probe (Abbott Molecular) (Fig. 4B). Thus, the maximum deletion region is a 10-megabase interval within 22q11.21 and 22q12.1 (Fig. 4C).

Chemotherapy with adriamycin and cisplatin was started at week 0. Soon after starting treatment, the tumor broke through the skin of the right arm, and the patient developed persistent fevers. We decided to proceed with forequarter amputation at week 3, rather than attempt further neoadjuvant chemotherapy before amputation (Fig. 1D). Due to massive tumor size, in addition to neurovascular compromise, limb-salvage resection was not an option. Histology revealed 100% tumor necrosis in the osseous component, but only 5% necrosis in the soft tissue component. After the amputation, the patient continued a 3-drug chemotherapy regimen consisting of high-dose methotrexate, adriamycin, and cisplatin. His calcium levels slowly increased to near-normal levels, which prompted multiple decreases in his calcium supplementation (Fig. 2). PTH levels normalized 12 weeks after the amputation. Currently, the patient is near the end of a standard chemotherapy regimen and continues to require 5.4 g of oral calcium glubionate and 1000 IU of cholecalciferol daily to maintain ionized calcium levels between 1.12 and 1.25mmol/L, just slightly below the lower limit of normal (nL 1.22 to 1.38). Urinary calcium levels remain undetectable.

DISCUSSION

We describe the first report of hypocalcemia in a patient with osteosarcoma and 22q11.2 deletion syndrome. Four previous cases of hypocalcemia in osteosarcoma have been reported.³⁻⁶ Common to all of these cases was the presence of widespread metastatic disease, all of which involved multiple bony lesions (Table 1). Many authors distinguish these cases as multifocal osteosarcoma, suggesting multiple simultaneous primary skeletal tumors, associated with extremely poor prognosis. Regardless of the terminology, the fact that all of the patients had extensive disease burden that precluded surgical resection is sufficient to account for their early demise.⁹

Another common feature in these cases was that serum calcium concentrations normalized rapidly after the start of IV calcium supplementation. This is not surprising, as deposition of calcium into primary or metastatic osteoblastic lesions should be a slow ongoing process. Homeostatic mechanisms that cause increased resorption likely lead to normal serum levels in most cases. In rare cases, when the body can no longer compensate, exogenous supplementation should result in rapid correction of serum levels.

22q11.2 deletion syndrome is a relatively common disorder, with an overall prevalence of 1 in 6000.¹⁰ In most cases, the deletions arise de novo; however, they can be inherited from a carrier parent. Thus, family history, genetic counseling, and parental cytogenetic testing as indicated, are recommended. The clinical phenotype is widely variable, and patients may present with congenital heart disease, dysmorphic facies, palatal anomalies, and

immunodeficiency. Hypocalcemia is present in 17% to 60% of patients with 22q11.2 deletion syndrome.⁷ Its etiology is generally accepted to be the result of hypoparathyroidism because of abnormal formation and/or function of the parathyroid glands due to abnormal development of the fourth and fifth pharyngeal pouches. Interestingly, hypocalcemia has been seen with low and normal PTH levels.¹¹ Most commonly, the hypocalcemia is transient, but can be recurrent, especially when a patient encounters increased metabolic demands, such as in times of stress.

The exact contribution of our patient's 22q11.2 deletion to hypocalcemia remains unclear. We believe that the combination of calcium deposition in the tumor and the 22q11.2 deletion-related predisposition to dysregulation of serum calcium levels led to refractory hypocalcemia. It is indeed noteworthy that the patient remains hypocalcemic despite a decreased tumor load, consistent with his genetic defect. On the other hand, it is also impressive that a patient with a 22q11.2 deletion had a PTH level of 290 pg/mL, a seemingly appropriate PTH response to the level of serum calcium at the time. One must assume though, that this PTH response was inadequate because his serum calcium remained low, which indicates that abnormal formation or function of parathyroid glands due to the 22q11.2 deletion may have played a significant role in the pathogenesis of hypocalcemia. The clinical data of this case are important for the understanding of the mechanisms of hypocalcemia related to constitutional 22q11.2 chromosomal deletions and point to a functional defect in addition to anatomic/embryologic causes.

Is there a connection between loss of genes in 22q11.2 and development of osteosarcoma? One possibility is that there is no relationship, and that our patient had 22q11.2 deletion syndrome and unfortunately also developed osteosarcoma. The fact that this is the first case report of both entities suggests that we have come across 2 rare, independent, disease entities. On the other hand, abnormalities that involve chromosome 22 have been reported in osteosarcomas. Bridge et al¹² examined 73 tumor samples from 51 patients and found that 15% had structural rearrangements of 22q11-13.

CHEK2 is one interesting gene in this region. Activated *CHEK2* is a checkpoint gene that stabilizes *p53*. *CHEK2* mutations are inherited in some Li-Fraumeni cancer syndrome families and it is also mutated in 4 of 57 (7%) sporadic osteosarcoma samples.¹³ Further molecular studies of our patient's tumor sample may reveal interesting findings that help us to better understand the pathogenesis of osteosarcoma.

Acknowledgments

Supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research and National Institute of Child Health and Human Development.

REFERENCES

1. Bielack SS, Kempf-Bielack B, Delling G, et al. Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol*. 2002; 20:776–790. [PubMed: 11821461]
2. Raskin P, McClain CJ, Medsger TA. Hypocalcemia associated with metastatic bone disease. *Arch Intern Med*. 1973; 132:539–543. [PubMed: 4742409]
3. Mahoney JP, Spanier SS, Morris JL. Multifocal osteosarcoma: a case report with review of the literature. *Cancer*. 1979; 44:1897–1907. [PubMed: 387210]
4. Chap LI, Mirra J, Ippolito V, et al. Miliary osteosarcomatosis with associated hypocalcemia. *Am J Clin Oncol*. 1997; 20:505–508. [PubMed: 9345337]
5. Gunawardena S, Chintagumpala M, Trautwein L, et al. Multifocal osteosarcoma: an unusual presentation. *J Pediatr Hematol Oncol*. 1999; 21:58–62. [PubMed: 10029815]

6. Soltani A, Hasani-Ranjbar S, Moayyeri A. Hypocalcemia as a presentation for multifocal osteosarcoma. *Pediatr Blood Cancer*. 2007 ahead of print.
7. Sullivan KE. The clinical, immunological, and molecular spectrum of chromosome 22q11.2 deletion syndrome and DiGeorge syndrome. *Curr Opin Allergy Clin Immunol*. 2004; 4:505–512. [PubMed: 15640691]
8. Bachrach LK, Hastie T, Wang MC, et al. Bone mineral acquisition in healthy Asian, Hispanic, black, and Caucasian youth: a longitudinal study. *J Clin Endocrinol Metab*. 1999; 84:4702–4712. [PubMed: 10599739]
9. Kager L, Zoubek A, Potschger U, et al. Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant cooperative osteosarcoma study group protocols. *J Clin Oncol*. 2003; 21:2011–2018. [PubMed: 12743156]
10. Botto LD, May K, Fernhoff PM, et al. A population-based study of the 22q11.2 deletion: phenotype, incidence, and contribution to major birth defects in the population. *Pediatrics*. 2003; 112:101–107. [PubMed: 12837874]
11. Choi JH, Shin YL, Kim GH, et al. Endocrine manifestations of chromosome 22q11.2 microdeletion syndrome. *Horm Res*. 2005; 63:294–299. [PubMed: 15995343]
12. Bridge JA, Nelson M, McComb E, et al. Cytogenetic findings in 73 osteosarcoma specimens and a review of the literature. *Cancer Genet Cytogenet*. 1997; 95:74–87. [PubMed: 9140456]
13. Miller CW, Ikezoe T, Krug U, et al. Mutations of the CHK2 gene are found in some osteosarcomas, but are rare in breast, lung, and ovarian tumors. *Genes Chromosomes Cancer*. 2002; 33:17–21. [PubMed: 11746983]

FIGURE 1.

A, X-ray of the right humerus. B, Computed tomographic scan of the right arm that also shows multiple metastatic nodules in the lung parenchyma. C, Bone scan demonstrates uptake in the right arm, pelvis, and lung fields. D, Photograph of the right arm immediately before amputation.

FIGURE 2.

Laboratory values. A, The graphs show the values for ionized calcium, magnesium, and phosphorus. B, The amount of IV and oral calcium supplementation required. C, Timing of chemotherapy and surgery. The patient requires 2 more months of chemotherapy to complete the standard regimen. A indicates Adriamycin; P, cisplatin; M, methotrexate; S, forequarter amputation.

FIGURE 3.

DXA scan. A whole body DXA scan revealed an overall Z-score for bone mineral density of -0.9 . However, 32% of the total body calcium was deposited in the primary tumor. When the right upper extremity was subtracted from the calculation, the Z-score was -2.85 , a value that is extremely low for the patient's age. DXA indicates dual energy x-ray absorptiometry.

FIGURE 4.

Fluorescence in situ hybridization analysis. A, A representative metaphase spread showing deletion of the TUPLE1 (22q11.2) probe (orange) from one chromosome 22. B, A representative metaphase spread and interphase nucleus demonstrate that 2 copies of the 3' and 5' portions of EWSR1 are present, unarranged, in both. C, A diagram of chromosome 22 shows the locations of TUPLE1, EWSR1, and ARSA. The maximum deletion region is a 10-megabase interval that encompasses TUPLE1 (22q11.21) and is centromeric to EWSR1 (22q12.1).

TABLE 1

Summary of Case Reports of Osteosarcoma and Hypocalcemia

Age	Sex	Presentation	Calcium	Course	Primary	Sites of Metastases	Treatment	Survival (mo)	References
23	M	Back pain	8.7 (9.0–10.5)	Unknown	Unknown	Entire skeleton, LP, LN, multiple organs	A, RT, chordotomy	2	3
26	F	Knee pain	7.9 (8.5–10.5)	Tetany resolved after IV calcium	Pelvis	Entire skeleton	APL, laminectomy, M	14	4
10	M	Knee pain	5.1 (8.5–10.5)	Resolved after IV calcium and calcitriol	Femur	Entire skeleton, LP, lungs	AP	1	5
16	M	Paresthesia	6.5 (8.5–10.5)	Resolved after 3 d of IV calcium	Femur	Multiple skull and spine, LN, abdomen	MAP	2	6

A indicates Adriamycin; I, ifosfamide; LN, lymph nodes; LP, lung pleura; M, methotrexate; P, cisplatin; RT, radiation therapy (normal levels).