Multiple Lytic Bone Lesions Mimicking Langerhans Cell Histiocytosis: A Case of Infantile Mendelian Susceptibility to Mycobacterial Disease due to STAT1 Deficiency

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We present the first infantile disseminated Bacillus Calmette-Guérin (BCG) disease case with STAT1 deficiency, which is manifested by multiple Langerhans cell histiocytosis–like osteolytic lesions. The diagnosis of BCG-induced osteomyelitis was not initially considered until the additional biopsy revealing granulomatous inflammation, a key pathological diagnostic component for mycobacterial infection.

Keywords. disseminated BCG disease; STAT1 mutation; Langerhans cell histiocytosis; osteolytic lesions.

The etiology of osteolytic lesions varies in children. FEGNOMASHIC (fibrous dysplasia [FD], eosinophilic granuloma [EG]; a form of Langerhans cell histiocytosis [LCH] with bone involvement), enchondroma, giant cell tumor [GCT], nonossifying fibroma, osteoblastoma, aneurysmal bone cyst [ABC], solitary bone cyst [SBC], hyperparathyroidism [Brown tumor], infection, chondroblastoma) represents the most common cause of osteolytic bone lesions. In this regard, <1% of cases have been proven to be caused by Langerhans cell histiocytosis (LCH) [1]. These osteolytic lesions have either a wide or narrow zone of transition and even more demonstrate a penetrative destruction pattern. There is a propensity for location in the calvarium, pelvis, ribs, and long bones such as the femur. Within long bones, LCH is typically metaphyseal or diaphyseal associated with periosteal reaction. Disseminated Bacillus Calmette-Guérin (BCG) infection is another cause of osteolytic bone lesions. Usually, the BCG-induced osteolytic lesions are found in immunocompromised patients, that is, patients with HIV infection or primary immunodeficiency. However, there are no specific pathognomonic radiological findings for these entities.

Here we demonstrated an infantile multifocal bone tuberculosis case with a total of 26 osteolytic lesions, mimicking LCH with bone involvement. An 11-month-old girl was suspected to have LCH due to multiple osteolytic lesions with tissue mass and was seen in the hematology/oncology department, referred by a pediatric orthopedist, on March, 2020. The patient was unable to raise her head, self-support by arms or hands, or roll over for 1 month. Physical examination did not reveal any palpable mass, ipsilateral extremity swelling, regional lymphadenopathy, or hepatosplenomegaly. No fever or chronic cough, weight loss, or tuberculosis (TB) exposure or contact was recorded. Building upon these features, a suspicion of neurologic or developmental disease was made. A whole-body bone x-ray and thoracic spinal computed tomography (CT) scan identified destructive vertebral lesions and a paraspinal mass, ruling out skeletal disease. At this point, an eosinophilic granuloma or LCH was suspected. Prominent red-brown nodules on the left axillary region were observed. Further laboratory studies revealed a high white blood cell count (20.3 × 10⁹/L, normal range 5−12 × 10⁹/L) with significantly increased eosinophils counts (1.4 × 10⁹/L, normal range 0.025−0.6 × 10⁹/L), moderate anemia (hemoglobin, 71 g/L, normal range 105−145 g/L), mild elevated platelet counts (523 × 10⁹/L, normal range 140−440 × 10⁹/L), and an increased erythrocyte sedimentation rate of 50 mm per hour. A lymphocyte subpopulation analysis was normal (Supplementary Table 1). Meanwhile, no active infection was identified by clinical testing such as bacteria culture, antibodies against Epstein-Barr virus, toxoplasma, respiratory syncytial virus, cytomegalovirus, herpes simplex virus, taenia solium, or Aspergillus spp. and Candida quantitative polymerase chain reaction assays. In addition, interferon-gamma release assay (IGRA) results were normal. Besides, bone marrow aspiration revealed increased eosinophils, without abnormal blasts or histocytes. Moreover, a radiology report suggested that the features were most likely to be LCH in light of multiple lytic, punched-out sections in the parietal and frontal regions, left tibia, ischium, scapula, ribs, and bilateral femurs and humerus (Supplementary Figure 1). Further to this, a spinal CT scan demonstrated multilevel noncontiguous destructive lesions involving the cervicodorsal to lumbosacral regions. The vertebral bodies and the posterior elements were affected in the thoracic spine, T2 and T3, and the lumbar spine, L1. The thoracic spine, T3, presented with extensive flattening of an entire vertebral body,
characterized by vertebra plana appearance. In addition, osteolytic lesions limited to the pedicular and posterior elements were visualized in involvement of the cervical vertebrae C6 and C7 and the thoracic vertebrae T4, T7, and T11. An extradural soft tissue mass was found in the posterior paravertebral regions, extending into the spinal canal at the level of T2-T4, T7, T11, and L1 and leading to spinal cord tissue compression at the T2-T4 level. Although the chest CT scan was normal, abdominal CT revealed multiple small speckle low-density shadows throughout the splenic parenchyma and a solitary low-density, $1.7 \times 1.3$-cm mass located at the left renal pelvis. All these radiological findings were further confirmed by magnetic resonance imaging (MRI) scan (Supplementary Figure 2). To systemically demonstrate the osteolytic lesions, we summarized and plotted these 26 bone lesion sites, as shown in Figure 1A.

Given that a provisional diagnosis of LCH was made, a CT-guided biopsy was thus performed on the right radius lesion. Histopathologic analysis revealed non-necrotizing granulomatous inflammation without Langerhans cell infiltration. An additional biopsy on the left distal tibia was performed but without clean conclusion. The result of BRAF genetic testing was negative using quantitative polymerase chain reaction and Sanger sequencing (Supplementary Figure 3). Subsequently, a pathology consultation revealed a non-necrotizing granuloma with an accumulation of multinucleated giant cells. These infiltrated histiocytes were characterized by epithelioid appearance with abundant cytoplasm, nuclei of a “horseshoe”-shaped pattern that were aggregated in 1 or both poles of the cell, and a peripheral rim composed of numerous epithelioid histiocytes, fibroblasts, and lymphocytes, suggesting a potential tuberculosis infection (Figure 1B). Further, the acid-fast bacillus stain for mycobacteria and Gomori methenamine silver stain for fungus were negative. Tuberculin skin was thus performed, and a strongly positive reaction (20 mm of induration, 48 hours of inoculation) was performed.

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**Figure 1.** A, Diagram of involved bones. A total of 26 bony sites were affected by disseminated BCG disease, as shown in the front and back of the whole-body skeleton. The bones highlighted are affected sites. B, Pathological evaluation of the left tibia osteolytic lesion. The microphotograph shows granuloma formation consisting of an abundant collection of multinucleated giant cells characteristic of abundant cytoplasm, nuclei of a “horseshoe”-shaped pattern and aggregated in 1 or both poles (arrow), and a peripheral rim composed of numerous epithelioid histiocytes, fibroblasts, and lymphocytes (H&E, 400x). C, STAT1 deficiency. A Sanger sequence was used to identify the STAT1 V450D mutation in the patient and her parents’ blood samples. D, Reduced IFN-γ-induced STAT1 phosphorylation despite normal levels of STAT1 production by immunoblotting. Western blot of a total protein extract from PBMCs from the patient and her parents control samples, which were probed for phosphorylated STAT1. Abbreviations: BCG, Bacillus Calmette-Guérin; H&E, hematoxylin and eosin; IFN-γ, interferon-gamma; PBMCs, peripheral blood mononuclear cells.
observed. To exclude the TB being contagious, tuberculin skin test and chest x-ray (CXR) were prescribed for all of the patient’s caregivers with normal results.

In the neonatal period, the patient received BCG (Chinese substrain Shanghai D2PB302, derived from Danish strain 823) inoculation immediately after birth. Within the first week of inoculation, she experienced severe dermatologic complications manifested by a red-brown pustule with pain, swelling, and erythema, which evolved into ulceration with drainage exactly at the inoculation site within the second week. One month after vaccination, a subcutaneous abscess and regional suppurative lymphadenitis occurred in the ipsilateral axilla. Three months of isoniazid (INH) monotherapy led to remission, but with permanent residual scarring at the left axillary region.

Given the BCG vaccine predisposition, Mendelian susceptibility to mycobacterial diseases (MSMD) was suspected, a rare congenital predisposition syndrome to infections caused by weakly virulent mycobacteria, such as BCG vaccines and nontuberculous environmental mycobacteria (EM). Moreover, we performed whole-exome sequencing (WES) to explore the genetic variants and identified a heterozygous STAT1 de novo mutation (c.1378A>G, p.N460D), which was confirmed by Sanger sequencing (Figure 1C). To reveal the effect of this mutation on STAT1’s function, phosphorylation of STAT1 was leveraged to compare the immune response of peripheral blood mononuclear cells (PBMCs) from this patient and her parents after treatment with 10 ng/mL of interferon gamma (IFN-γ). PBMCs with p.STAT1^{N460D} responded poorly to IFN-γ, with 50% of STAT1 being phosphorylated as compared with cells with wild-type STAT1 (Figure 1D). Furthermore, we evaluated protein kinase C (PKC)–induced intracellular IFN-γ production by flow cytometry. Four hours of stimulation with phorbol myristate acetate (PMA) and ionomycin (ION) did not impair IFN-γ production (Supplementary Figure 4).

Taken together, these results indicated that the impaired IFN-γ response incurred by a de novo p.STAT1^{N460D} mutation might be the underlying genetic cause. Due to poor BCG response to pyrazinamide and the spinal instability of this case, 2 months of intensive therapy with INH (5 mg/kg daily), rifampin (RIF; 10 mg/kg daily), amikacin (10 mg/kg daily), and linezolid (10 mg/kg twice a day) was prescribed to maximize the treatment response and prevent further neurologic impairment. Thanks to the intensive anti-TB therapy, the symptoms quickly and clinically improved, as evidenced by independent sitting and rolling over independently 2 weeks later. After that, she received a total of 15 months of maintenance therapy (INH and RIF). Fortunately, the osteolytic lesions had completely resolved after 18 months, and no any sequelae or side effects were observed later on (Supplementary Figure 5).

As of the writing of this article, this patient has been continuously followed for 28 months.

Mycobacterium tuberculosis is the most common cause of mycobacterial osteomyelitis and arthritis worldwide. However, in recent years, M. bovis skeletal infections incurred by intravesical BCG have been occasionally reported [2]. It is extremely challenging to distinguish between acquired or vaccine-induced mycobacterial osteomyelitis and LCH so far. Systemic evaluation with caution is highly warranted to make precise differential diagnoses, such as the lesion’s type of margin, pattern of bone, destruction, type of periosteal reaction, and presence of an associated soft tissue mass (Table 1). Though systemic evaluation could provide insightful diagnostic information, biopsy remains the gold standard. Unfortunately, in this case, neither bone lesion culture was performed because the treating physician did not consider mycobacterial infection. BCG is administered worldwide to prevent tuberculosis, with an excellent safety profile. However, complications caused by BCG itself occasionally occur, as a result of direct spread from the administration site [3–7]. In particular, “BCG-osis” due to disseminated infection following BCG vaccination is a typical MSMD feature, which is characterized by insufficient IFN-γ production and/or incomplete IFN-γ response. Mutations of 19 genes responsible for MSMD have been reported, which are associated with IL12/IL-23 or ISG15-INF-γ circuits [8–12]. To our knowledge, the STAT1 pathogenic variant in this patient has not been reported either as a mutation or polymorphism in the National Center for Biotechnology Information database, Ensemble database, Single Nucleotide Polymorphism Database, Exon Variant Server, or Human Genome Variation Database.

### Table 1. Radiologic Imaging Characteristics of Mycobacterium- and LCH-Related Osteo-Lesions in Children

| Imaging | Langerhans Cell Histiocytosis | Mycobacterial Osteomyelitis |
|---------|-------------------------------|---------------------------|
| Common involvement sites | Flat bones | Weight-bearing bones/joints |
| No. of osteo-lesions | Single | Multiple |
| Spinal lesion | Thoracic followed by lumbar and cervical | The lower thoracic or upper lumbar |
| Skip lesion | Uncommon | Uncommon but usually contiguous |
| Vertebral body | Vertebra plana | Anterior wedging, body collapse |
| Pedicular and posterior element | Uncommon | Common |
| Disc space | Normal or slightly widened | Narrowing |
| Skull | Common | Rare |
| Long bones | Diaphysis/metaphysis, lytic lesion | Metaphysis, rare epiphysis lesion, a ballooned appearance |
| Soft-tissue mass | Extension from adjacent bone marrow | Abscesses with calcification |

Abbreviation: LCH, Langerhans cell histiocytosis.
In conclusion, we present an infantile disseminated BCG case with identified genetic immunocompromise, characterized by as many as 26 osteolytic lesions, and established regional BCG inoculation reaction and disseminated tuberculosis disease. These clinical manifestations confused her physicians. An awareness of these potential etiologies is needed for timely and correct prospective diagnostic testing and treatments. We need to highlight the importance of consideration of all possible tests when a biopsy is done to avoid additional biopsies. Childhood skeletal tuberculosis is often delayed or even missed, which can potentially cause irreversible harm. Therefore, physicians should keep in mind that TB cannot be neglected while working on any infective pathology. Finally, infantile disseminated TB should be included in the differential diagnosis of MSMD among countries with regular BCG vaccination.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. Y.H. and H.Z. analyzed and interpreted the data and wrote the manuscript; X.S. collected the data and performed the follow-up. C.L. performed the experiments. Y.H., H.Z., W.G., and P.W. carried out the clinical treatment, and W.X. obtained and analyzed images. All authors have critically reviewed the manuscript and approved the submission.

Patient consent. The patient’s written consent was obtained for publication of this report. The work was approved by the institutional review board (IRB) of Guangzhou Women and Children’s Medical Centre (2020-58).

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