NEEDLE(S) IN THE HAYSTACK – SYNCHRONOUS MULTIFOCAL TUMOR INDUCED OSTEOMALACIA

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Authors' contribution

All of the authors were involved in the clinical care of the patient, and contributed to the writing of the manuscript.

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A 49-year-old man was referred to our endocrine unit in 2012 with a 15-year history of widespread incapacitating bone and joint pain, which was now causing marked insomnia. Physical examination revealed proximal muscle weakness, waddling gait, kyphosis and a right metacarpal swelling. Previous biochemical investigations had revealed a low serum 25-OH vitamin D of 16 ng/mL [reference range (RR) 30-100], elevated serum alkaline phosphatase (391 U/L; RR 53-128), normal serum corrected calcium (9 mg/dL; RR 8.5-10.5) and a low serum phosphorous level (1.8 mg/dL; RR 2.5-5). He had previously undergone extensive radiological and rheumatological investigations, which were 'inconclusive'. Plain X-ray studies had revealed multiple rib and vertebral fractures. MRI of the spine showed diffuse sclerosis of the vertebral column and pelvic bones. A Tc-99m-MDP bone scan performed in the year 2000 had shown increased uptake over the ribs bilaterally (Fig.1A), which was initially reported as suspicious for metastases. However, extensive cross-sectional imaging with CT and MRI failed to identify an underlying primary lesion. Prior to the current presentation he was treated with multiple analgesics, calcium, phosphorous and calcitriol preparations. However, none of these resulted in any improvement in his symptoms.

As there were no other systemic/localizing features, we considered the possibility of a primary metabolic bone disorder. A repeat phosphorus level was persistently low (1.86 mg/dL), with an inappropriate 24-hour urinary phosphorus of 745 mg/day (RR <1000) and the % Tubular Reabsorption of Phosphate (% TRP) off supplements was 0.68 (RR>0.8). 25-OH vitamin D (32 ng/mL) and parathyroid hormone levels (49 pg/mL; RR 14-72) were normal, but 1,25-$(OH)_2$ vitamin D was inappropriately low (3.5 pg/ml; RR 19.6-54.3). In view of this, plasma C-terminal Fibroblast growth factor 23 (FGF-23) level was measured (Immutopics, USA), which was grossly elevated at 3088 RU/mL (RR 0-150). A whole body ^{18}F-FDG-PET scan showed focal tracer uptake on the radial and dorsal aspect of the head of the right second metacarpal (SUVmax=2·5) (Fig.1B, C, D). Histological analyses of the surgically-resected metacarpal lesion revealed a

phosphaturic mesenchymal cell tumor (PMT), mixed connective tissue variant with negative FGF-23 and somatostatin receptor subtype (SSTR) 2A immunostaining (Fig.1E, F, G). Despite the latter, FGF-23 levels declined rapidly to 246 RU/mL on day 6 postoperatively, with progressive symptom relief, confirming a diagnosis of tumor-induced osteomalacia (TIO).

In view of persisting (albeit considerably improved) pain and mild hypophosphataemia (2.4 mg/dL), a repeat ^{18}F-FDG-PET scan was performed in 2013 and showed low-grade residual uptake over the head of the right second metacarpal. After careful discussion with the patient, a ray amputation of the index finger was performed with histology confirming complete excision of the residual tumor. Following the second operation, FGF-23 levels dropped to 152 RU/mL, but the hypophosphataemia persisted. His pain improved although he described on-going mild symptoms. Several months later the patient reported an increase in the intensity of his bone pain. He had persistent hypophosphataemia with rising FGF-23 levels (224.5 RU/mL). In 2015, a whole body ^{68}Ga-DOTANOC-PET scan was performed, which revealed no uptake at the site of the previous surgery. However, a left nasal cavity lesion (2.9 x 2.0 cm), (Fig.1H, I) with avid tracer uptake (SUVmax 3.79) was seen. Retrospectively, this lesion could be seen on the ^{18}F-FDG-PET, although at that time the relatively low level of tracer uptake was not felt to be significant. However, axial reconstruction clearly shows the lesion (Fig. 1J). Further review of the more historical imaging suggested that this was also probably visible on the original Tc-99m-MDP bone scan (Supplementary Fig.A, B). Transnasal endoscopic resection of the nasal lesion revealed a predominant, phosphaturic mesenchymal cell tumor (Fig.1K, L) with negative FGF-23 immunostaining but positive SSTR 2A immunostaining (Fig.1M), suggestive of a multifocal TIO. Post-operatively FGF-23 levels returned to normal within 48 hours of surgery (64.6 RU/mL), and currently remain within the reference range (50.3 RU/mL) at 3 months, with normalization of phosphorous levels (4.2 mg/dL) and, for the first time, complete resolution of his symptoms.

Tumors that secrete phosphaturic factors/phosphatonins (e.g. FGF-23) are usually benign and the predominant cause of TIO or oncogenic osteomalacia. As in our patient, the resulting hypophosphataemia can cause debilitating pain. Complete resection of a unifocal tumor typically leads to full resolution of symptoms. However, localization of these tumors is often difficult due to their small size and diverse locations (limb extremities to the head, including soft tissues, bones, sinuses, brain). Systematic physical examination, combined with cross-sectional and functional imaging is essential for localization. Amongst functional scans, some studies have shown somatostatin receptor ligand based imaging [e.g. ^{111}Indium-Octreotide SPECT, (1) ^{99}Tc-HYNIC-TOC SPECT(2) and Gallium based ^{68}Ga-DOTA-TATE PET(2-5)] to be better for identifying PMT in comparison to other imaging modalities such as ^{18}F-FDG-PET. In a large case series, Chong et al showed that amongst 19 pathologically confirmed TIO subjects, 18 were octreo-SPECT scan positive [sensitivity 0.95, specificity 0.64, positive predictive value (PPV) of 0.82 and a negative predictive value (NPV) of 0.88] and 14 out of 16 confirmed TIO subjects were FDG-PET positive (sensitivity 0.88, specificity 0.36, PPV of 0.62 and NPV of 0.50). In addition, in a small number of subjects ^{68}Ga-DOTA-TATE/DOTANOC PET has localised TIOs that were not previously identified with ^{18}F-FDG-PET (2) and ^{111}Indium-Octreotide SPECT imaging (3). In our case the intense brain uptake of ^{18}F-FDG-PET obscured the lesion in the nasal cavity (Fig.1B), and it was only on retrospective review of axial imaging that the lesion was noted (Fig.1J).

Occasionally, even with the best efforts to localize these tumors, they may remain elusive (‘a needle in the haystack’). In this scenario, a combined imaging approach with structural and nuclear imaging affords the best opportunity for localization. Moreover, in the setting of multiple suspicious lesions on initial imaging, or with a possible multi-focal TIO similar to our case, an ideal initial approach retrospectively could have included biopsy of both the lesions and/or

selective venous sampling of FGF-23 around the sites of the lesions. Selective venous sampling has been shown to help in tumor localization by using an FGF-23 venous concentration ratio between the venous drainage of the tumor bed in comparison to the general circulation (6). In this way, selective venous sampling can differentiate between a culprit lesion and other incidental lesions thereby avoiding unnecessary surgery (6).

Previous reports have always noted complete normalization of hypophosphataemia and FGF-23 levels with successful surgery. In our case, biochemical normalization did not happen in spite of complete surgical excision of the metacarpal lesion following second surgery. Although multifocality is extremely rare, and has only previously been described in four patients (7-10), persisting symptoms and failure of FGF-23 levels to normalize after complete excision of the metacarpal lesion prompted a detailed re-evaluation of the case, and the adoption of more sensitive imaging in the form of Ga-68 DOTANOC PET. It is likely that if the latter had been used as the preferred imaging modality at the time of his referral to our service, then a structured approach as outlined above, combining selective venous sampling and biopsy would potentially have led to earlier identification of the multifocality, although still have required two separate surgical procedures.

This image highlights the importance of a multimodal, perseverant approach towards the investigation and treatment of patients with persistent unexplained hypophosphataemia and chronic bone pain, and serves as an important reminder of possible multifocal disease in unresolved cases of TIO.

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Figure legend

(A) Tc-99m-MDP Bone scan showing increased tracer uptake at multiple sites; **(B)** Whole body ^{18}F-FDG-PET scan revealing FDG avid soft tissue thickening over the radial and dorsal aspect of the head of the right second metacarpal (SUVmax=2.5); Fused ^{18}F-FDG-PET & CT of the right hand in **(C)** sagittal and **(D)** coronal planes; **(E)** Histological examination of metacarpal lesion showing diffuse proliferation of bland tumor cells in a patternless arrangement around vessels **(F)** with bluish smudgy matrix; **(G)** Vimentin positivity on immunohistochemistry (IHC); **(H)** Whole body Ga-68-DOTANOC-PET scan showing avid tracer uptake within left nasal cavity lesion (2.9 x 2.0 cms, SUVmax 3.9); **(I)** Fused Ga-68 DOTANOC PET & CT scan head; **(J)** ^{18}F-FDG-PET SPECT CT Head axial imaging demonstrates the left nasal cavity lesion with a low SUV uptake (SUV Max = 2.1); **(K)** Histological examination of nasal lesion showing bland tumor cells with fibromyxoid stroma, heamangiopericytomatous pattern of vasculature [arrow]**, (L)** smudgy matrix [arrow]; **(M)** IHC shows strong positive staining for somatostatin receptor subtype 2 A (SSTR2A); **(N)** Graph depicting the trends in FGF-23 levels over time.

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