Ozlem Goker-Alpan:

Welcome everyone to another year of our series on lysosomal disorders. This is a series focused on the several systems that are related to the management of the conditions associated with lysosomal disorders. So today's presentation presents the skeletal system involvement in lysosomal diseases. So let's start with the skeletal involvement in lysosomal disorders, and first we want to think about our sponsors from Takeda and Ultragenyx for this series.

These are the disclosures for myself and for the speaker today, Dr. Ravi Kamath.

I would like to have a very brief introduction, because our speaker today is phenomenal, and then he has many information about the bone involvement in lysosomal diseases. Skeletal system is actually one of the biggest system probably after skin that is involved. It's a bigger system, and also, it is involved mainly in lysosomal disorders as the primary presentation such as a skeletal disorder. These are mainly the mucopolysaccharidoses and related disorders, such as multiple sulfatase deficiency and ML I, II and III.

But also, skeletal system is also involved in other disorders, such as Gaucher disease, which we are going to be focusing on today, either as a developmental manifestation or as an acquired finding. The presentation may, as I said, either can be developmental, that's a progressive kyphoscoliosis, as you see in a child with type 3 Gaucher disease, or it could be acquired, as you see here, the cystic lesions. Actually, they are Gaucheromas in Gaucher disease.

That pathophysiology, as suggested, is multiple. Obviously, the accumulated substrates, such as GAGS or sphingolipids, can disrupt the chondrocyte or osteoblastic function. Then, the inflammatory cytokines could be upregulated. That promotes osteoclastogenesis. Also, the altered endochondral ossification causes dysostosis multiplex, which is the telltale sign of MPS. Also, the cartilage and synovium could be affected in Gaucher disease. Obviously, that is, there is bone marrow infiltration, and these cytokines can lead to the bone density abnormalities that is common, actually, in lysosomal disorders.

So there is a bone modeling and remodeling imbalance, and there are reduced osteoprotegerin levels that exacerbate bone resorption. There is a novel finding that we published that allows the abnormalities in bone marrow adipose tissue interactions such as sclerostin is, I think, is a major player in the bone density abnormalities in lysosomal disorders. How we manage the skeletal involvement, obviously, it requires an interdisciplinary approach. We're going to hear from a expert radiologist today, but there are many interdisciplines are involved here, such as the geneticists, or the metabolic specialist, the physical therapist, orthopedic surgeon, anesthesiologist.

One person, usually geneticist, coordinates the diagnostic and therapeutic intervention, also long-term monitoring. That is, the orthopedic surgeon can come into, actually, as the primary caregiver in some disorders, such as the MPSs, because they do require interventions. So the person can collaborate with the team, also. So the prognosis actually is dependent on the early surgical and medical intervention and, obviously, the extent of skeletal damage. The team ensures comprehensive management and for pre and post-surgical optimization. It is important, actually, to provide these patients for supportive care, including the orthotics, physical therapy, and pain management can be a primary ... can come into the primary picture before or after the surgical intervention.

So before I go into further detail, I would like to introduce you today's speaker, who is Dr. Ravi Kamath, and he's a colleague and a friend. We have been working together for over a decade now on radiology of the lysosomal disorders. Dr. Kamath Is the section chief of Muscular Skeletal Radiology at Fairfax Radiological Consultants in Fairfax, Virginia. He has a stellar resume. He graduated magna cum laude from Harvard in 1997 and has a PhD from Cambridge. Cambridge, he has multiple fellowships, including a molecular by informatics, and after he pursued his medical degree, he worked as an intern in internal

medicine and a resident at diagnostic radiology at MGH, Mass General Hospital. So before, that is, we lose our further time, Dr. Ravi Kamath for today's presentation.

Ravi Kamath:

Thank you very much for that introduction. So today, as Dr. Goker-Alpan stated, I'm going to speak about bone complications in lysosomal storage disorders, and these are my disclosures, which are the same ones that she shared earlier. So as an outline for this talk, I'm going to start by introducing lysosomal storage disorders. Dr. Goker-Alpan already did that, so we can just go over those briefly. Then, I'm going to talk about the of skeletal abnormalities in the LSDs and I'm going to group these into three broad categories.

So the first is the MPSs, which share a common clinical presentation in the musculoskeletal system as dysostosis multiplex. I'll speak briefly about pyknodysostosis, which has a different musculoskeletal presentation, and then I'll speak in a more extended fashion about Gaucher disease. I'll talk a little bit about the management of bone abnormalities and LSDs, both medical and surgical, and then talk about some recent developments on the imaging side.

So let's get started. So lysosomal storage disorders, as most of you probably know, are individually rare disorders, but as a group, they can affect up to one in 5,000 live births. They result from an accumulation of glycosaminoglycans or single lipids in the lysosomes. They're usually caused by a genetic mutation resulting in a failure to manufacture or process an enzyme needed for the breakdown of a specific product. This can affect the skeleton by causing the accumulation of substrates in macrophages or connective tissue cells. The most common of these are Gaucher disease and the mucopolysaccharidoses, and the pathophysiology, as Dr. Goker-Alpan stated, likely involves a combination of space occupation, inflammation, and epigenetic factors.

So as I stated before, I'm going to speak about these in three different groups. So first, I'm going to talk collectively about the mucopolysaccharidoses as well as some other similar disorders like the mucolipidoses, multiple sulfatase deficiency, because these have a similar clinical presentation with dysostosis multiplex. Then, like I said, I'm going to speak briefly about pyknodysostosis, and then I'm going to talk about Gaucher disease.

So MPS. MPSs is a group of 11 LSDs that are caused by defects in the catabolism of glycosaminoglycans, and the specific enzyme deficiency involved in each and the storage material that accumulates is known for each of these. Each MPS is a progressive multi-system disorder with skeletal manifestations, a major cause of morbidity. Although the primary enzyme deficiency and accumulated substrate have been identified for these disorders, as I stated, the mechanism, underlying disease symptoms, and skeletal manifestations are still a matter of discovery. Also, the presentation of these disorders is clinically very heterogeneous. Skeletal disorders are the presenting system for most of the MPS types, and these findings, known as dysostosis multiplex, result from the defective and endochondral and membranous ossification and bone maturation.

So dysostosis multiplex is a variable constellation of skeletal abnormalities and changes that occur during bone growth and development. All types of MPS show dysostosis multiplex, but to varying degrees and varying severities. Dysostosis multiplex is also a feature of other mucolipidoses and lysosomal storage diseases, as I stated earlier. So I'd like to now just go over the radiographic findings of dysostosis multiplex. So first, this is a lateral view of the skull, and one of the clinical presentations is macrocephaly, which is just having a large skull, also a J-shaped sella turcica.

So the sella turcica is the bony structure that contains the pituitary gland, and normally, it's shaped like a semicircle or like a U shape. In these patients, they have a J shape, so it's sort of elongated. They also

present with a thickened calvarium with a widened diploic space. In the thorax you can see short, thick clavicles as well as flattened broad ribs, which are called paddle or oar-shaped ribs, and also a short sternum. On these images, you can also see some of the long bone findings, such as medial humeral notching in the proximal humeri.

In the spine, these patients present with platyspondyly, which means flattened vertebrae, and they have beaked or notched vertebral bodies with posterior scalloping and odontoid dysplasia. So in these cases, I show you examples here. For example, the first two patients have Hunter disease or MPS I. So these patients have this posterior vertebral scalloping, as I showed you earlier, and these patients also have a characteristic inferior beaking, so beaking sort of closer to the inferior end plate of the vertebra.

In contrast, Morquio and Hurler's have the same platyspondyly and posterior notching. Morquio also has this inferior beak just like in Hunter, but Hurler, you can see, has, more characteristically, a central beak of the vertebra. So these morphologic differences are characteristic of these disorders. Patients can present with odontoid dysplasia or hypoplasia. For example, in this patient, normally, you should be able to see the odontoid process here arising from the superior part of the anterior C2 vertebra, and here, it's completely absent. And so this patient is at risk for atlantoaxial instability.

In the pelvis, patients have rounded iliac wings that taper distally, and here, again, we can see some of the findings in the long bones, as well, with these dysplastic epiphyses and long femoral necks. Here's another example of the long bone findings in addition to those that we showed earlier. So they can have these short, thick diaphyses with long femoral necks, the humeral notching that we talked about earlier, and they can also have fragmented or hypoplastic epiphyses.

Here's another example where you can see some fragmentation of the epiphyses and these short, thickened diaphyses. This genu valgum deformity is also something that is typical of MPSs. In the hands and feet, there are short, thick metacarpals and metatarsals that are tapered or point proximally. They can have irregular hypoplastic carpal and tarsal bones.

So in MPS, there's generally a disturbance in linear growth that results in short stature, and this typically starts at around 18 months of age. Often, patients have little linear growth in the skeleton after eight years of age. The morphologic bone abnormalities, some of which we discussed earlier, include macrocephaly, spinal deformities with kyphoscoliosis, gibbus, and odontoid dysplasia, plus dural and ligament thickening. When those two features are combined, that can cause compression of the spinal cord. Patients can develop chest deformities with pectus carinatum and rib deformities, which can cause a form of restrictive lung disease. They also develop progressive arthropathy with age, which results in contractures and joint destruction.

So here's an example of a patient with MPS, and, as stated previously, these people can have pronounced spinal deformity with kyphoscoliosis and a gibbus deformity, as you can see here. So this is something that can result in compression of the spinal cord, as you can see here, which can eventually require surgery to correct that and prevent lower extremity weakness. Here is an example of a patient with MPS who has these short, flattened vertebrae and also has ligament thickening, which also results in central canal stenosis in the cervical spine and resulting cord compression.

In the chest, patients then have this pectus carinatum deformity with these broad, flat ribs that we talked about, and as stated previously, these can cause restrictive lung disease. Morphologic bone abnormalities can occur with progressive arthropathy with age. Here's a patient who is an older patient in their thirties with Morquio who now has severe osteoarthritis in both hips, and on the MRI, you can see, essentially, the cartilage is completely denuded. There's osseous remodeling, subcortical cysts, marginal osteophytes. So this is end-stage osteoarthritis at a very young age. Here is, again, another

patient with Morquio with this genu valgum deformity, and you can see the cortical deformity and sclerosis here on the radiographs, which is also readily apparent on MRI.

So now, I'm going to speak briefly about pyknodysostosis. So pyknodysostosis is a rare autosomal recessive bone dysplasia that is caused by deficiency of cathepsin K, which is necessary for osteoclast function. This typically presents with short stature, especially the limbs, diffuse increased bone density, delayed closure of the cranial sutures, which can result in frontal and occipital bossing. Their hands are short and broad with nail hypoplasia, and they can develop long bone fractures with minimal trauma due to bone fragility.

One of the most well-known people with pyknodysostosis is the French painter Toulouse-Lautrec, and this is a photograph of him showing this short stature and short limbs that's characteristic in these patients. In the head, they have a marked delay in suture closure, which can result in frontoparietal bossing and calvarial thickening. The hands, they can have short, stubby fingers with partial agenesis of the distal phalanges. Sometimes, this is termed as distal acroosteolysis, but it's really more an agenesis of the distal phalanges. They also present with delayed bone age. In the long bones, they have this diffuse osteosclerosis with very, very thickened cortices and very narrowed medullary cavities, and they also present with abnormal fractures due to bone fragility.

Okay. So now, we're going to talk about the imaging of skeletal abnormalities in Gaucher disease. So Gaucher disease is the best-studied and best-understood lysosomal storage disorder. It's an autosomal recessive disorder resulting from the deficiency of the enzyme acid beta-glucocerebrosidase. There are almost 200 known gene mutations, but the most common mutation, which is the N370S mutation, accounts for about 50% of the cases. The prevalence is about one in 40,000 to one in 60,000, and there are three types with the type 1 non-neuronopathic form being the most common.

So as we stated previously, Gaucher disease results from a deficiency in the acid, the beta-glucocerebrosidase enzyme, which is an enzyme which is responsible for conversion of glucosylceramide to ceramide and glucose. The deficiency of this enzyme results in an accumulation of glucosylceramide, which is a glycolipid that accumulates in the Gaucher cells of the monocyte and macrophage system. This can result in visceral abnormalities like hepatosplenomegaly and thrombocytopenia anemia, but can also result in skeletal complications which occur in greater than 80% of patients with Gaucher.

That includes infiltration of the dark marrow by Gaucher cells, so-called dark marrow, loss of bone mineral density resulting in osteopenia and osteoporosis, focal deposition of Gaucher cells, resulting in Gaucheromas or lytic lesions in the bone, pathologic fractures, osteonecrosis, or the death of bone, and chronic bone pain or acute bone crises. The deficient activity of glucocerebrosidase during development results in slow growth and delayed puberty in a majority of patients in addition to the skeletal and visceral complications that we discussed earlier.

So what are the imaging findings of Gaucher disease? So first, we'll look at plain radiography or X-ray. So one of the characteristic findings of Gaucher disease is this Erlenmeyer flask deformity, which is a smooth tapering of the distal femoral metaphysis as opposed to a more curved appearance in a normal individual. This is named after its appearance, which is similar to an Erlenmeyer flask that's used in chemistry.

Fractures can be seen radiographically. This is an example of a compression fracture of the L1 vertebra in the spine. But by X-ray, we don't know whether this is an acute or chronic abnormality, but we can see the fracture. Osteonecrosis, or death of bone, can occur. The most common location is here in the femoral head, and on X-ray, it manifests as an area of geographic sclerosis with central lucency. So you can see this. This is the patient's right side, is abnormal with osteonecrosis as compared to the normal

left side. Lytic bone lesions can occur from focal deposition of Gaucher cells, and that results in osteolysis or destruction of bone, which appears as a radiolucent area on the X-ray.

Patients who have Gaucheromas can develop pathologic fractures due to the weakening of bone at the site of the bone lesion. MRI is also a very valuable tool to characterize Gaucher disease in bone. On MRI, bone marrow infiltrated with Gaucher cells typically appears hypointense or dark on both T1 and T2-weighted images, which is why it's called dark marrow. The affected bone marrow can also be T2 hyperintense, which can also be seen with superimposed osteonecrosis, marrow infarction, or infection, which are other phenomena that can occur in Gaucher disease. Focal bone lesions, so-called lytic lesions or Gaucheromas, may also be seen in some patients.

So what does this look like on MRI? So here are two different patients with Gaucher disease. Here on the left side of your screen is a patient with relatively mild Gaucher disease. Again, you can see this Erlenmeyer flask deformity in the distal femurs, and this patient has mild T1 hypointense bone marrow signal, which is most pronounced here in the femoral necks and intertrochanteric regions. So this is a very mild form of Gaucher's, of dark marrow in Gaucher disease.

Here, on the right-hand side of the screen, is an individual much more severely affected, and what's interesting about this particular person is that this person has very asymmetric disease. You can see that this patient's right femur has some dark marrow, but much worse on the left side. The left side also has osteonecrosis of the femoral head with articular surface collapse and destruction of the joint and has focal deposition of Gaucher cells with either chronic marrow infarcts or possibly lytic bone lesions.

The lumbar spine also can show varying degrees of severity. So here on the left side of your screen is someone with mild dark marrow infiltration diffusely throughout the lumbar spine. In the middle is someone with more severe involvement. So you can see how the bone marrow signal here is darker than in the first patient. One characteristic appearance is that sometimes, these patients can have relative preservation of fat adjacent to the basivertebral veins in the posterior vertebrae. This is a characteristic appearance that can occur in some patients, but you can start to see, even in this patient, how there's some morphologic changes that are occurring in some of the vertebrae but not all.

Finally, here on the far right is a patient with very severe disease, so not only with diffuse heterogeneous dark marrow infiltration, but also with an abnormal morphology of the vertebrae with biconcave vertebrae. Sometimes, these are called H-shaped vertebrae or codfish vertebrae, because they have almost the shape of the letter H or a fish with a fish tail. So this is the most severe appearance of the spine in Gaucher disease.

Dark marrow has a very typical presentation. So here's a patient with symmetric dark marrow infiltration in the distal femoral metaphyses and the proximal tibial metaphyses, and there's sparing of the epiphyses. So this is a characteristic geographic deposition of dark marrow that occurs, and when the dark marrow worsens, it advances in a predictable fashion. When it improves, either with age or response to therapy, it also regresses in exactly the reverse sequence.

Patients with Gaucher disease can present with bone crises, which is acute bone pain. Sometimes, when these patients present and have an MRI, you can see abnormal marrow edema in the femoral head and neck, and this patient also has a joint diffusion on that side. So this is a reactive phenomenon to the changes undergoing in the bone.

Bone crisis is seen in up to one third of patients with Gaucher. These patients can present with acute severe skeletal pain. This may be accompanied by fever and abnormal lab results. These patients also may show subtle periosteal elevation on radiographs, marrow edema on MRI, or the imaging may be completely normal. This can be difficult to distinguish from subchondral fracture or osteonecrosis in the early phase.

It's important to note that bone pain in general is common in Gaucher disease, and often, it does not have an imaging correlate. So subjective bone pain does improve with therapy. So you can see here on the top is an example of patients who are treated, and over time, their subjective bone pain does decrease. It's also important to note that Gaucher patients can have bone or joint pain that's not related to Gaucher just like anyone else.

So MRI is particularly useful for the characterization of bone findings in Gaucher, because it can see things in a much earlier phase than radiography. Dark marrow, in particular, is something that is visible on MRI but is not visible at all on X-ray. So here's an example of a patient. You can see on the right-hand side of the screen, this is an MRI, a coronal T1 weighted image showing dark marrow infiltration again in the femoral neck, intertrochanteric region, and proximal diaphysis with relative sparing of the femoral head epiphysis and the trochanteric apophysis. But when you look at the X-ray of the same patient, there's really no geographic abnormality that you can see that matches up with the dark marrow. The patient does have abnormal bone with a thin cortex and cortical tunneling indicative of osteopenia or osteoporosis, but the dark marrow itself cannot be directly visualized on X-ray.

Here is a different patient with fractures of two of the thoracic vertebrae. We can see those fractures on X-ray, but we don't know whether they're acute or chronic. Now, when the patient has an MRI, we can see that there's marrow edema in those vertebrae suggesting that these fractures are either acute or subacute. Here's a patient with an X-ray of the hip, which looks normal, but on MRI, you can see geographic subcortical sclerosis in the subchondral bone of the femoral head, which is consistent with osteonecrosis. This is actually the definition of grade 1 osteonecrosis, which is that it is visible on MRI, but it is not visible on radiographs.

Here's a patient who has diffuse marrow infarcts throughout the distal femur and proximal tibia and even in the proximal fibula. I think these are well characterized as these areas of geographic sclerosis, but the problem is, again, you don't know whether these are acute or chronic and if they could be a cause of pain. However, when the patient has MRI, you can see that there is, in fact, marrow edema and even soft tissue edema associated with these marrow infarcts in the distal femur, suggesting that these are possibly acute or at least acute on chronic. Whereas the marrow infarct in the proximal tibia looks indolent with no adjacent edema.

This is the patient that I showed you earlier who has lytic bone lesion or Gaucheroma in the tibia, and when you get the MRI of this patient, you can see that actually, the bone lesion is much more extensive than is apparent on the radiographs. So here, it just looks like a part of the bone is involved, but in fact, the only part of the lesion that you can see on the X-ray is the part where it has broken through the cortex. Most of the bone lesion is not visible. So because this lesion involves so much of the bone and weakens the bone to such an extent, this is a lesion that, perhaps, an orthopedist might want to intervene on.

DEXA, or dual-energy X-ray absorptiometry, is an important tool for measuring bone density in patients with Gaucher. Typically, DEXA is done in the femoral neck or in the lumbar spine. Usually, we get a lot of information from a DEXA, because we get a report that looks like this. But really, the most important features that we look at are the T-score and the Z-score. So the T-score is comparing the bone density of a patient with an average bone density of a young, healthy adult of the same sex.

The WHO characterizes these as, essentially, normal if you're within one standard deviation of normal, as low bone mass or osteopenia if you're between one and two and a half standard deviations below normal, or osteoporosis if you're greater than two and a half standard deviations below normal. The Z-score, in contrast, compares a person's bone density with the average bone density of other people of the same age, sex, and body type. This is defined as normal if it's within two standard deviations and abnormal if it's greater than two standard deviations below normal.

So in summary, the three main imaging modalities that we use in Gaucher disease are X-ray, MRI, and DEXA. X-ray and MRI are both useful for characterizing a wide variety of pathology in Gaucher, although MRI is much more sensitive and allows for better characterization of those abnormalities. MRI is uniquely available, uniquely suited to evaluate dark marrow in patients with Gaucher, and DEXA is especially suited for assessing bone mineral density in those patients.

So now, I'd like to talk a little bit about the management of bone abnormalities in LSDs, and then we'll focus in on the role of the orthopedic surgeon. So the management of LSD bone disease is similar to the management of LSDs In general. The treatment is really focused on the underlying cause of the disease, replacing or enhancing the missing or deficient enzyme or shifting the balance between substrate and product.

So examples of this would be enzyme replacement therapy, so literally infusing the deficient enzyme in order to replace its function, substrate reduction therapy, so shifting the balance between the substrate and the product to prevent the accumulation of the metabolite, pharmacologic chaperone therapy, which is basically helping to stabilize a malfunctioning or misfunctioning enzyme in order to augment its function. Hematopoietic stem cell transplantation has a very limited utility for certain disorders, and there are new and emerging therapies, including gene therapy and genome editing, which are currently being tested.

So there are current treatments available for different types of MPS. Again, stem cell transplantation is really for MPS I, has a limited role, but not for any of the other disorders. There is enzyme replacement therapy for several of the mucopolysaccharidoses, and for Gaucher disease, there's a variety of treatments available, including multiple types of enzyme replacement therapy and substrate reduction therapy. There are different types of gene therapy that are also undergoing clinical trials.

As Dr. Goker-Alpan stated before, these efforts to aid or replace the enzyme are supplemented by supportive care targeting specific aspects of the disease presentation. So, for example, patients may take anti-inflammatory agents to reduce some of the bone inflammation that's associated with these disorders. They may take medications to either increase or inhibit bone formation, depending upon which is the underlying problem in bone in that disorder, orthopedic procedures to deal with severe or irreversible skeletal complications or to improve quality of life, and other medications and treatments for the non-skeletal disease manifestations.

So studies do show improvement in symptoms and quality of life with treatment for patients with LSDs. Some skeletal problems are refractory to therapy, and, of course, there are some skeletal complications that are irreversible once they have formed. Bone changes in response to therapy have been most thoroughly studied for Gaucher.

So here's an example of a patient with Gaucher disease who received therapy, and as you can see, here on the left is the baseline. Then, this is re-imaging of the same area in the distal femur after one year, two years, and three years of therapy, and you can see that there's a progressive diminution of the amount of dark marrow in the distal femur from year to year. Roughly 60 to 70% of the patients demonstrate this improvement in bone marrow on MRI with medical treatment, and I should state that studies so far have shown that the magnitude of change in terms of enzyme reduction therapy and substrate reduction therapy are pretty comparable, both in the 60 to 70% range.

Here's another example of a different patient, looking at the lumbar spine. So here, again, on the left, you can see at baseline, there's diffuse dark marrow infiltration. At two and a half years, there's been considerable improvement on therapy, and then, even another one and a half years later, there's been further improvement in the bone marrow.

Likewise, other disease parameters can improve with therapy. Here's an example looking at the Z-score as measured by DEXA. So you can see that with successive 12-month intervals, the bone marrow density has continue to improve in the lumbar spine, in the hip, and in the proximal femur. You notice that in the radius and ulna, it doesn't make quite so much of an improvement, perhaps because that's not a load-bearing part of the body. Also, this has been demonstrated to occur in a dose-dependent fashion. So patients who are getting higher doses of enzyme replacement therapy have greater improvements in their DEXA Z-score as compared to patients who are on lower doses of therapy.

So these are a set of imaging recommendations from the International Collaborative Gaucher Group Gaucher Registry. They recommend that patients who either are not on therapy or who have already achieved their therapeutic goals get imaging surveillance roughly every one to two years, patients who have not achieved their therapeutic goals get imaging surveillance once every year, and patients who have either a dose change, or a medication change, or significant clinical complication might need customized intervals for their imaging with potentially even more frequent imaging follow-up.

Okay. So now, we're going to talk a little bit about the role of the orthopedic surgeon. So orthopedic care in MPS really focuses on the severe and irreversible complications and to improve quality of life. Interestingly, the most common surgical procedure that's performed in patients with MPS is carpal tunnel and trigger finger release. This can sometimes even be done in children, because it often presents very early.

Soft tissue surgery for release of joint contractures is sometimes performed, but the outcomes of those have been inconsistent and relatively poor. Spinal fusion may be needed to treat thoracic kyphoscoliosis, as we showed earlier, and also atlantoaxial instability. Patients may also require corrective surgery for hip subluxation, genu valgum, and angle valgus by requiring osteotomies of the relevant bones. When patients reach end stage osteoarthritis, as we showed earlier, they may require hip or knee arthroplasty.

So here are some examples of surgical interventions that were performed in patients with MPS. So here's a patient, again, with very severe kyphoscoliosis and gibbus deformity. Here, you can see, on the CT, severely-deformed bones, some sclerotic changes in the bones with endplate deformities, and this results in severe narrowing of the central canal. And so this patient ended up requiring spinal fusion in order to stabilize the spine and to decompress the cord.

Here's a patient who presents with odontoid dysplasia or aplasia, so it's essentially an absent odontoid process. As a result, the C1 ring is not constrained and can sublux relative to C2. So here, again, you can see, on MRI in that same patient, that there is a considerable narrowing of the central canal, in part due to the thickening of the soft tissues and the ligaments in that area. So this is treated with a occipitocervical fusion, which you can see here. And so on the MRI, you can see that in addition to the fusion, they also do a surgical decompression to create space to relieve that cord compression, and then the fusion is to stabilize and prevent subluxation.

Here's a patient with Morquio who underwent hip osteotomies in order to correct a severe hip dysplasia. So again, osteotomy and K-wire fixation of both acetabula and also osteotomy and fixation of both proximal femurs. This is a patient who presented with genu valgum and had surgery in order to correct the genu valgum using hemiepiphysiodesis of the femur and tibia, which you can see here, so the screw fixation, which allows us to straighten the femurs, and, you can see, with a much improved mechanical result.

In Gaucher disease, as for MPS, the procedures really focus on the severe and irreversible skeletal complications and to improve quality of life. Patients who develop femoral head osteonecrosis may eventually require hip arthroplasty when the joint begins to collapse. Surgery may be required to assess

structural abnormalities of the spine, especially ones that occur due to spinal compression fractures. When patients develop fractures in the extremities, they may require fixation. Sometimes, patients can develop Gaucher bone lesions, which require prophylactic fixation due to risk of pathologic fracture. It's important to remember that Gaucher patients have additional risks due to bone weakness, delayed healing, and bleeding problems.

So here's an example of a patient who has osteonecrosis, actually in both femurs. So in the left femur, it's obviously much more pronounced with sclerosis, and articular surface collapse, and developing osteoarthritis. Here on the right side, also, you can see this geographic sclerosis in the femoral head. So there is osteonecrosis on this side, as well, and with very subtle cortical deformity here immediately suggesting that there may be some very early articular surface collapse. This patient eventually did go on to have bilateral hip replacements.

Here's a patient who has a large lytic bone lesion in the proximal femur and developed a non-displaced pathologic fracture through that bone lesion which required fixation. Patients with Gaucher disease often have osteoporosis or osteopenia, and they can be given bisphosphonates in order to strengthen their bones. One of the unfortunate side effects of bisphosphonates is that they can develop what are called atypical femur fractures, which occur, characteristically, along the lateral cortex of the proximal femoral diaphysis.

As you can see here, this is focal area of cortical thickening, which is an incompleted atypical femur fracture. On the other side, you can see that this patient had something similar on that side, but this fracture actually completed. So now, it broke all the way through the bone, and that required a surgical fixation of that fracture. Here's another patient with a lytic bone lesion in, this time, in the radius, and again, because this lesion is so big, and it's thinning the cortex, the decision was made to fix this fracture. You can see here, here's another lytic bone lesion in the distal humerus.

So I'd like to talk to you a little bit about the more recent developments in imaging in Gaucher. So in LSD bone imaging, there are some new and modified imaging techniques. They're kind of along the lines of what we've seen before, but these are kind of new spins on these techniques that allow us to better characterize the disease and progression. So, for example, as an alternative to DEXA, looking at the cortical thickness of bone on radiographs or using a special, a high-resolution CT using MR spectroscopy for Gaucher disease and the use of artificial intelligence for analyzing MR images. So I'll just talk about these briefly.

So the radiographic cortical thickness is basically a measurement of the cortex on X-ray just in this way done here. You essentially measure the total thickness of the bone at the level of the proximal diaphysis in a specific location, and then you also measure the thickness of the medullary cavity. If you subtract those two, that gives you the total cortical thickness of the femur. According to this study, they showed that this cortical thickness index was a stronger predictor of fracture risk than DEXA, which is performed at various sites.

So here's an example of a patient with normal bone mineral density here on the left and a Gaucher patient with decreased bone mineral density. You can see if you compare the thickness of the cortex here in the proximal femoral diaphysis on a normal patient versus a patient with Gaucher, the patient with Gaucher obviously has a much thinner cortex with a much wider medullary cavity. It is thought that that thickness reflects the overall weakness of the bone. Now, it's important to note that the bone consists of both cortical bone and also trabecular bone. And so this does not measure the thickness or the density of the trabecular bone, and it only measures the cortical bone.

So this is a useful piece of information that can be obtained without requiring an additional test or piece of information. So this is potentially useful, especially in resource-poor areas where you have access to

radiography, but maybe you don't have access to DEXA. One of the nice things about this particular measurement is that it's something that seems like it should be easily amenable to analysis by artificial intelligence and machine learning algorithms, which could automate this process and make it much simpler and more standardized.

Another example of a tool to measure bone mineral density or bone strength is high-resolution peripheral QCT, also called HR-pQCT, and this uses a non-invasive imaging technique of the distal radius and the distal tibia to provide volumetric measurement of bone mineral density and also a 3D assessment of bone microarchitecture. The way that it works is it's a machine with a small opening, and essentially the body part that's being imaged is inserted into that opening, so, for example, here, looking at the wrist, or here, looking at the distal tibia.

This tool is essentially, it's a variation on a CT, but done at low dose and just of that focal area in order to minimize radiation exposure. So this tool allows CT examination of structural properties and geometry of the cortical and trabecular bone, because CT is a technique that requires, specifically, measurements of bone density. Some studies have shown that this has higher sensitivity for detecting skeletal changes as compared to DEXA, including showing earlier changes and changes over shorter time intervals.

So what does this look like? So essentially, this technique allows you to make these three-dimensional models of bone. So, for example, here on the top is a control subject looking at the distal tibia and the distal radius, and here on the bottom is a subject with osteopenia or osteoporosis who developed an insufficiency fracture. So here on the top, you can see what the normal trabecular structure looks like, and here on the bottom, you can see how the trabeculae are resorbed with rarefaction. There's also greater spacing of these trabeculae from each other. So this is what happens when you lose bone mineral density.

Here, likewise, in the radius, you can see the same phenomenon, with large open spaces and increased space between the trabeculae. In addition, you can see areas where the cortical bone is also thinned. So this is a potentially useful tool for measuring bone mineral density, but, of course, the weakness is that it does require a higher level of radiation compared to radiography, for example. It also requires a special imaging test in order ... a special piece of equipment in order to take this measurement. Here's another example of a patient with Pompe disease on the left-hand side of the screen and osteopetrosis on the right-hand side of the screen. You can see how in the patient with osteopetrosis, the trabeculae are much thickened and much more densely-packed as compared to a patient with Pompe.

Proton MR spectroscopy is a variant on MRI which basically allows us to better characterize the chemical composition of bone. So one of the difficulties in evaluating bone in Gaucher is that it's difficult to differentiate the dark marrow, which is the marrow infiltrated with Gaucher cells, from hematopoietic red marrow, which is a normal physiologic type of bone marrow that occurs in young patients, especially in children. It's this physiologic process of red marrow conversion, as the red marrow converts to yellow marrow with age, that can make it more difficult to assess for Gaucher and dark marrow. MR spectroscopy provides a direct quantitative comparison of the chemical composition of the bone marrow and allows us to compute a so-called fat fraction, which can be used to quantify the amount of bone marrow involvement.

So here's an example of a six-year-old girl with untreated Gaucher disease. You can see that this is a T1 weighted image, right, because the fat is very bright. Essentially, there's no fat at all in the bone marrow. So this is basically just diffuse dark marrow. You can see here on the spectroscopy, there's a very tall peak for water, which is the red marrow or dark marrow, and there's essentially no fat peak at all. So the femoral neck fat fraction here is less than 0.01.

So one of the important things to remember is that as patients age, it's normal that both patients with ... control subjects and patients with Gaucher disease have an increased fat fraction with age. So it doesn't completely ameliorate the problem that we have differentiating between dark marrow and red marrow with age, but at least, if this is done in enough patients, we can perhaps develop some index measurements that will allow us to compare Gaucher patients to a population of control patients.

Here's an example of a 15-year-old patient who, at baseline, had, basically, diffuse dark marrow. So this would be very abnormal for a patient who's 15 to have, essentially, no fatty marrow or yellow marrow at all. After one year of treatment with enzyme replacement therapy, this patient now has a lipid peak that you can see here, which is now about one-third the height of the water peak. After two years, you can see that the lipid peak is about half the height of the water peak, so again, showing improved yellow marrow on imaging and corresponding improvement in the fat fraction on MR spectroscopy.

So this is a useful tool. It can actually quantify the bone marrow burden so you get a fat fraction, which is a number. So you can actually find differences even when the MRI maybe doesn't show tremendous qualitative differences. This is very useful for tracking response to therapy. So it's important to remember, as I stated, that normal marrow conversion can affect the results due to an expected increase in fat fraction with age. And so really, some larger studies are needed to better characterize the normal progression in normal individuals and the progression in individuals with Gaucher so that we can better understand how these fat fraction measurements can be used.

Finally, this is an example of artificial intelligence or deep learning to assess MRI images. So this group used a deep learning-based quantification system in order to segment areas of abnormal bone that are seen on MRI. So, for example, here is an image of the lumbar spine, and you can see here, there are areas of geographic osteonecrosis in the bone. The machine learning tool was able to accurately identify, here in orange, the areas that are affected by osteonecrosis and to separate them from these blue areas that are not affected by osteonecrosis. Similarly, in the femurs, you can see that the machine learning tool is able to segment these areas that are involved in osteonecrosis from the normal background bone marrow.

So why is this useful? Well, first, it can improve standardization of imaging interpretation. So you have the tool basically helping to define what is normal and what is abnormal. That can be especially helpful if the person who's interpreting the MRI is not very familiar with Gaucher. Second, it can be useful for evaluating bone architecture and for quantifying the size of bone lesions. So if they can be segmented, then they can be measured, and you can actually measure whether these lesions are increasing in size and the overall amount of bone that's being involved with bone lesions. It can also be used to augment scoring systems. So the ability to segment abnormal bone from normal background bone marrow is very important as a first step toward being able to automate some of the quantitative and semi-quantitative scoring systems that we have for MRI and Gaucher.

So today, we've talked briefly about the lysosomal storage disorders, their characteristic presentation of MPS with dysostosis multiplex. We talked briefly about pyknodysostosis and its unique presentation, and we talked in greater extent about Gaucher disease and about the different imaging modalities that we use. We talked about the management of bone abnormalities in lysosomal storage diseases, both medical and surgical, and we talked about some of the recent imaging developments. So at this point, I'll throw it back to Dr. Goker-Alpan, and we can take any questions.

Ozlem Goker-Alpan:

Thank you, Dr. Kamath, for a very detailed and great presentation. So there are a few questions in the chat. I want to start it to continue with the pediatric imaging, because it says you said it could be tricky. I don't know when we are going to realistically, we'll have the MR spectroscopy to understand the bone

marrow involvement in children. So what are some guidelines for assessment of red marrow, and the receding of it, and then whether the marrow is infiltrated by Gaucher cells? So what are some general guidelines for children for MRI?

The other question I have is obviously, the ICGG guidelines are quite old now. It is almost actually with the data of the origin of when ERT was established 25 years ago, and it's not always applicable to irradiate children every year. So with the data we actually collected over 10 years, so what are some gut feeling? How frequently we need to look for what? Do we need to get X-rays every year in these children or not for assessing, like, Erlenmeyer flask deformities?

Ravi Kamath:

Okay. So in terms of bone marrow in pediatric patients, so yes, very difficult. I think that in a static presentation, meaning you have one set of images to look at, I think it's very difficult to determine whether there's dark marrow or not. For me, for example, I have a series of normal patients that I've collected. So I have a normal standard that I look at anytime I'm looking at a patient of a certain age, and when I'm looking at a patient with Gaucher, I compare that bone marrow signal that I see in that patient to a normal individual of that age. And so to me, if it looks like it deviates significantly from that, then I would favor that that's abnormal.

Secondly, we know that the regression of red marrow occurs in a predictable fashion over time. And so again, if we see something that deviates from that, then I would be concerned that there could potentially be involvement either with dark marrow or with, potentially, a bone lesion or some other bone complication in that area. Now, when you have multiple sets of images over time, I think it's easier to tell whether the bone marrow is, the signal is getting worse or whether it's getting better.

Of course, again, you have the problem that if a young patient is getting therapy, and that patient is obviously also increasing in age, you would expect, with age, the amount of dark signal on the MRI to decrease. And so it's difficult to know how much of that decrease is due to marrow conversion and how much of it is due to improvement with therapy, again, without just a sort of a gestalt view by looking at age-matched controls and to understand what the bone marrow should look like at that age. But it is very, very difficult.

In terms of surveillance, yes, I agree. Those are old guidelines. I think, really, at this point, we kind of understand that patients with Gaucher present very heterogeneously, and everyone is different. There are some patients who need imaging surveillance much more than others, because their disease seems like it's very stable. In children, I think the main thing is that we want to make sure that they don't develop any of these irreversible complications that can potentially cause them to have a lifelong problem.

Erlenmeyer flask deformity, that's something that we can see on MRI. I don't think people necessarily need to have an X-ray for that, but when we characterize their bone marrow and just make sure that ... So we can, on MRI, we can look at the dark marrow infiltration. We can look at any of the bone lesions, like marrow infarcts, lytic bone lesions, and also osteonecrosis. And so really, I think the MRI is the best tool to look at children, and the main thing is just, how far apart can you space it? How comfortable are you in terms of patient's clinical presentation? Every year? Every two years?

I think it sort of depends on the individual patient, and I guess the other factor would be, in a very young child, obviously, if a patient cannot have an MRI without sedation, then that's another factor you have to keep in mind. But once patients get older, hopefully, they don't require that, and you can do the MRI without such the additional risk of the sedation.

Ozlem Goker-Alpan:

So what would be the ideal age to start surveillance with an MRI?

Ravi Kamath:

Yeah. I mean, I think, again, it sort of depends. So if you, if ... radiography, and you don't see any abnormality, then I think it's okay to start a little bit later. If you think you might see something on the MRI, then you need to start earlier, and whether ... So for me, there's usually an age somewhere between six and nine years when patients can reliably be in the tube and cooperate with the exam, depending on the maturity of the child.

And so I think at that point, that would be the appropriate time to start and take a look at the bone marrow. If you're going to do it before then, I think it would sort of require me to have something else that I see on the X-ray or something that would make me concerned, or if the patient has a lot of bone pain or something like some unusual clinical presentation.

Ozlem Goker-Alpan:

We have some, actually, seen some consults. They were doing MRIs on the little newborn babies, actually, so we don't do that.

Ravi Kamath:

Yes.

Ozlem Goker-Alpan:

The reason is it is very hard to understand what is the normal red marrow. I mean, it is hard.

Ravi Kamath:

Exactly. At that age, I mean, babies have mostly red marrow, and so it would be extremely difficult to determine whether a baby had any dark marrow or not.

Ozlem Goker-Alpan:

Let's start with the discussion, then, with the impact of the therapy. Obviously, this is before and after ERT now, and so now, the ERTs, we would like to start as early as it gets. Could we actually compare the effects of the treatment like MPS, where there's a cartilage and bone disease, versus Gaucher disease, and do we see any differences between the impact of the ERT in these kids, MPS versus Gaucher disease?

Ravi Kamath:

Yeah. So it's a little bit different, because with Gaucher disease, we're looking at this phenomenon of red marrow, which is something that really can sort of advance and regress. The changes in the bone in MPS lean more toward the sort of irreversible types of changes. So there are more morphologic changes in the bone that once you have them, they're there. What you're trying to do is prevent those very severe things like the end stage osteoarthritis and the gibbus deformity.

So those are things that if you diagnose early, and you treat early, you can, well, at least it's thought that you can ameliorate some of those symptoms in some patients or some of those disorders in some patients. But it's more difficult, because you only have one patient. I don't know if people have looked at

this at the population level, but I think it's something that's very difficult to do. And so I think always, early diagnosis and early treatment helps, but because you can't ... There's no imaging biomarker in the same way that the dark marrow works in Gaucher. Unfortunately, it's not as simple or straightforward to do monitoring in patients with MPS.

Ozlem Goker-Alpan:

Okay. I guess this concludes our discussion today, and I would like to thank the audience for their attendance. Before we conclude, I would like to still remind them, that is our, also, the sponsors, and also complete the program evaluation at the end, which is going to come at the end of the program. Thank you.

Ravi Kamath:

Thank you.