

**Exchange Grant Report**  
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**Understanding the *in vivo* bone remodeling alterations in patients affected by Gorham-Stout disease.**

**Background of the project**

Gorham-Stout disease (GSD) is a very rare disorder characterized by extensive and progressive osteolysis and angiomatous proliferation, without new bone formation. The first description of a patient affected by GSD was in the 1838; since then only ~200 patients were reported. The shoulder and the pelvis are the common sites of involvement; however skull, humerus, scapula, sternum, ribs, pelvis and femur can be affected. Patients display pain, functional impairment and swelling of the affected regions. When ribs, scapula or thoracic vertebrae are affected, development of chylothorax from the extension of lymphangiectasia into pleural cavity or via invasion of the thoracic duct can occur. Moreover, bone infection and subsequent septic shock, spinal cord involvement and paraplegia due to vertebral lesions have been reported. Without surgical intervention, the morbidity and mortality rate is very high.

The diagnosis of GSD is challenging and it is usually performed by exclusion criteria. Indeed, to define a proper diagnosis it needs to rule out neoplastic processes, infection, and metabolic and endocrine disorders. For the diagnosis many examinations are used. Blood tests are usually normal in GSD patients, with the possible exception of alkaline phosphatase levels, that could be high. Additionally, a diagnostic role could be played by plain radiographs, bone scan, computed tomography and magnetic resonance imaging (MRI). However, the results from bone scan and MRI are variable. All these procedures are useful, but the diagnosis must be confirmed by histopathological analysis of the lesions, that reveals evidence of local bone progressive resorption, angiomatous tissue and absence of cellular atypia.

The genetic alterations involved in the etiopathogenesis are not entirely known. Indeed, preliminary work performed by Prof. Lorenzo's group showed genetic imbalance in GSD patients, but the molecular mechanisms underlying GSD disease and the excessive bone resorption must be identified. Since in GSD patients a lymphatic and vascular proliferation within bone is observed, it was suggested that lymphatic and blood endothelial cells, in addition to macrophages, secrete TNF $\alpha$  and IL-6 that stimulate osteoclast differentiation and function.

Due to its rarity, there are no setted guidelines for the treatment and management of GSD. The therapeutic options for the patients are based on drugs treatment, radiation and surgery. Several pharmaceuticals have been used to treat GSD including anti-VEGF-A antibody, interferon alpha 2b, Bevacizumab, propranolol, steroids, vitamin D, calcitonin and bisphosphonates, but they are not resolute.

**Why laboratory was visited**

I decided to visit the laboratory headed by Prof. Schinke to improve my knowledge on

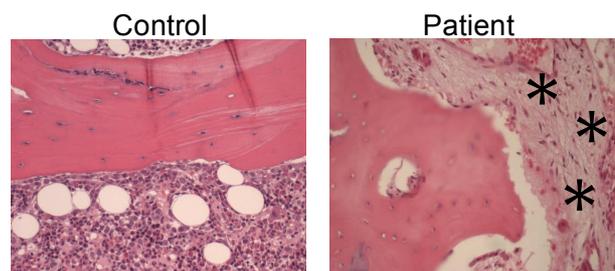
histomorphometric analysis and to perform analysis of patient and control's bone biopsies that we collected in our hospital. Particularly, histomorphometric analysis of osteoclasts and osteocytes have been performed to identify the mechanisms leading to progressive osteolysis in GSD. Since our *in vitro* preliminary results showed a reduction of Phospatase and TENsin homolog (PTEN) expression in GSD osteoclasts, immunohistochemical analysis was performed to evaluate PTEN expression in bone biopsies.

### **Skill learned and results**

During my stage at the Osteology and Biomechanic laboratory in Hamburg, I learned the procedures for the embedding of calcified and undecalcified human and murine bone biopsies, the stainings and the correct procedures to perform histomorphometric analysis. Under the excellent guide of Prof. Thorsten Schinke, I performed histomorphometric and immunohistochemical analysis.

Bone biopsies from 6 controls and 6 patients were stained for Hematoxylin and Eosin, CD68, CD31 and PTEN and histomorphometric analysis of osteoclasts, osteocytes and vessels was performed.

Histological analysis revealed fibrous tissue in bone marrow stroma of GSD patients (Figure 1).



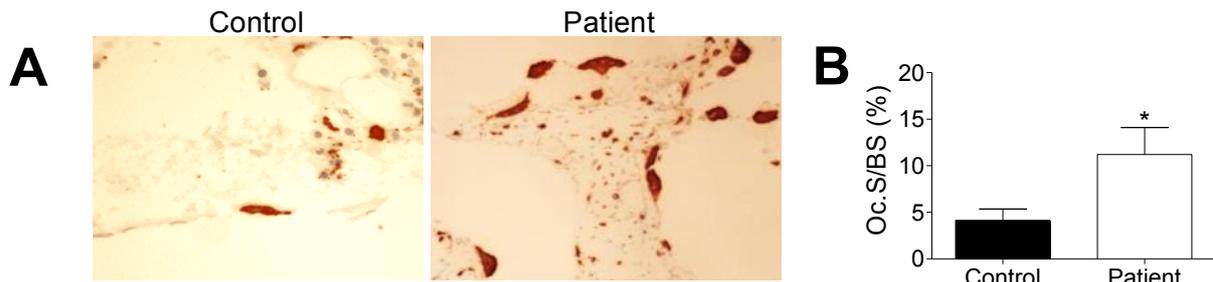
**Figure 1.** Hematoxylin and Eosin staining of patient and control's bone biopsies. Original magnification 10X.

Immunohistochemistry for the endothelial-specific antigen CD31 revealed an increase of vessel size and area (Figure 2 A-B) in bone biopsies isolated from patients compared to controls.



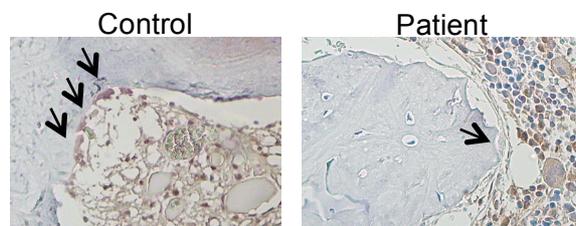
**Figure 2. A)** Immunohistochemical analysis of CD31 for patient and control's bone biopsies. Original magnification 10X. **B)** Histomorphometric analysis of CD31+ vessel area per bone marrow area. \*p<0.05 vs control

CD68 staining analysis showed an increase of very active osteoclasts as confirmed by high levels of Osteoclast Surface/Bone Surface (Oc.S/BS) (Figure 3 A-B).



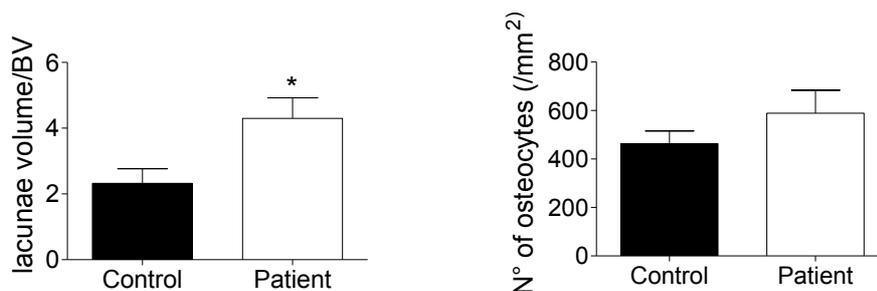
**Figure 3. A)** Immunohistochemical staining of CD68 of bone biopsies from control and GSD patient. Original magnification 20X. **B)** Histomorphometric analysis of osteoclasts surface on bone surface. \* $p < 0.05$  vs control.

Furthermore, the immunohistochemical analysis of PTEN revealed a reduction of this protein in GSD osteoclasts. (Figure 4).



**Figure 4.** Immunohistochemical staining of PTEN of bone biopsies from control and patient. Original magnification 20X.

As the osteolysis in Gorham-Stout patients is rapid and progressive, we evaluated the possible involvement of osteocytes in bone erosion. Moreover, GSD patients displayed larger lacunae without difference of the osteocyte number. (Figure 5).



**Figure 5.** Histomorphometric analysis of osteocytes lacunae volume and osteocytes number. \* $p < 0.05$  vs control.

## Discussion

Gorham-Stout disease is a very rare disorder with unknown etiology, diagnosis based on exclusion criteria and no setted guidelines to treat patients. Understanding the mechanisms involved in bone remodeling alterations in this pathology will be helpful to improve the diagnosis procedures and to identify new therapeutic approaches. The opportunity to visit the laboratory of Prof. Schinke was useful for my research to complete the *in vivo* analysis of patients bone biopsies and to match it with our *in vitro* experiments.

This disease is characterized by progressive osteolysis of bone and by angiomatous proliferation. Indeed our histomorphometric analysis revealed an increase number of very active osteoclasts confirming the increased ability of GSD-Peripheral Blood Mononuclear Cells to differentiate into mature osteoclasts. Moreover, the increase of VEGF-A levels in patient sera revealed by ELISA assay could explain the larger vessels revealed by CD31 staining of patients bone biopsies. As the bone resorption could also be mediated by osteocytes, we evaluated the role of these cells in the progressive osteolysis of GSD. Indeed it was demonstrated that osteocytes could resorb bone under appropriate stimuli including PTH and glucocorticoid stimulation, and lactation. Histomorphometric analysis of patients bone biopsies showed an increase of osteocyte lacunar size with no difference of cell number, suggesting an increase of osteocytic perilacunar resorption.

To understand the molecular mechanism involved in this disease, we performed a large gene expression analysis of GSD-osteoclasts. Interestingly, we found that a major modulation of genes implicated in PTEN pathway. Therefore, we performed immunohistochemical analysis of PTEN that confirmed the results obtained from gene expression analysis.

All this data, obtained under the great technical guidance in Hamburg, will be useful for us to complete our study regarding the mechanisms involved in bone remodeling alterations in GSD. We have data supporting the idea that a primary cell autonomous defect is involved though in this disease an important role is also played by systemic factors.