UNIVERSITÀ
DI SIENA
1240

Dipartimento di Scienze Mediche, Chirurgiche e Neuroscienze

Dottorato in Medicina Traslazionale e di Precisione

XXXVIII° Ciclo

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Cardiac Involvement in Paget's Disease of Bone: Echocardiographic Characterization and Genetic Correlations

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FILIPPO PIRROTTA
16/12/2025 09:14:57 UTC+0100

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Anno accademico di conseguimento del titolo di Dottore di ricerca
2024/2025

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CHAPTER 1: INTRODUCTION

1.1. PAGET DISEASE OF BONE

Paget's Disease of Bone (PDB) is a common chronic disorder of bone metabolism characterized by focal areas of excessive and rapid bone breakdown and formation [1]. These abnormalities disrupt normal bone architecture and result in enlarged and deformed bones in one (monostotic form) or more (polyostotic form) regions of the skeleton.It represents the second common osteometabolic disease after osteoporosis and it's considered a high bone turnover disease. A chaotic turnover between reabsorption and bone apposition leads to an enlargement and deformation causing an impairment in bone architecture and biomechanical inefficiency with an inadequacy in functional loading. Alterations in bone structure determine different symptoms such as local pain, fractures, osteoarthritis, nerve compression and other complications. [2]. Pagetic bone is also the site of intense metabolic activity with an increase in bone vascularization.

The affected skeletal sites are generally asymmetric and most frequently include the pelvis, spine, skull, femur and tibia (one or more of these skeletal sites are affected in up to 90% of cases) [3].

Described for the first time in 1876 by Sir James Paget, in England,[4] PDB remains not curable for a long time until the advent of bisphosphonates therapy.

The prevalence of disease increases with aging, affecting approximately 1% of individuals over the age of 50 and reaching up to 5% by the eighth decade of life in some countries. Several observations derived from radiological or necropsy investigations showed a high prevalence in whites of European origin, particularly in Great Britain, [5] followed by Australia, New Zealand, and the northeastern United States, countries with high rates of immigration of people of British origin in the 19th and 20th centuries. [6]

In contrast, the prevalence of the disease has been reported to be very low in Scandinavian regions, Asia, and Africa. [7]. There are also geographical areas with a

high prevalence of the disease, such as Lancashire in England, the district of "La Cabrera" in Spain[8], and the rural areas of Avellino and Caserta in Italy [9].

In PDB, the principal cell involved is the osteoclasts, while osteoblasts are normal [1]. Pagetic osteoclasts are significantly increased in both number and size, and it presents an are hyperreactive to various osteoclastogenic factors such as 1,25-dihydroxyvitamin D, tumor necrosis factor (TNF-α), or RANKL
[10]. [11].

Indeed, osteoclastic precursors produce high amounts of cytokines, especially interleukin 6 (IL-6), involved in the pro-inflammatory state, and TAF12 RNA polymerase II, a TATA box-binding protein (TBP), a VDR coactivator. The bone marrow microenvironment also appears to be abnormal and it has a greater capacity to induce osteoclast formation than the normal bone marrow microenvironment [12].

1.2. CLINICAL MANIFESTATIONS AND COMPLICATIONS

Skeletal involvement

In 25-40% of cases, the disease presents in a monostotic form, generally involving long bones, and in only 10% of cases it is symptomatic, with a good response to antiresorptive therapy. In 60-75% of cases, the disease presents in a polyostotic form, affecting more than one skeletal region and more frequently associated with neurological and vascular complications.

Bone deformation, enlargement and alterations in microarchitecture lead to an increased risk of **fractures**.

Pain localized at the level of the affected bone is the most common symptom that brings a patient with PDB to the attention of a physician [13]. When PDB reaches the end of a long bone, the cartilage can degenerate, causing **osteoarthritis**, a common complication of the disorder [14].

Neurological symptoms such as headache, dementia, cranial neuropathies, cerebellar dysfunction, myelopathy, cauda equina syndrome, and radiculopathies are common in particular in polyostotic form and occurring in 4-10% of PDB patients [15].

When PDB involves the skull, facial deformation (*facies leonina*) and hearing loss are the most common signs [16] [17]

Extraskeletal complications

Metabolic disorders, such as hyperuricemia, gout, chondrocalcinosis, hypercalcemia and/or hypercalciuria with nephrolithiasis, and a higher prevalence of primary and secondary hyperparathyroidism are common in PDB patients naive for treatment and in patients with long duration of the disease

Neoplastic degeneration of the pagetic bone is another complication of long-standing PDB. The most common malignant tumors are osteosarcomas (1% of pagetic patients,more frequent in pagetic compared with general population), while in the benign forms the most common is the Giant Cell Tumor (GCT) [18]. Considerable familial and geographic clustering has often been described for GCT with a percentage of cases reaching 50% in PDB families originating from Southern Italy who present a mutation in the ZNF687 gene [19.].Cardiovascular complications, including high-output heart failure, due to increased blood flow to the patellar tissue. In some series, a high incidence of vascular and cardiac valves calcifications has been found [20]. Cardiac alterations will be treated in detail below.

1.3. DIAGNOSTIC TOOLS

Increased remodeling, a hallmark of the disease, is expressed by an increase in bone turnover markers. Serum calcium, phosphate, parathyroid hormone (PTH) and 25-OH-vitamin D (25-OH-D) concentrations are generally normal. Serum total alkaline phosphatase (ALP) is the most widely used and recommended biochemical marker for the diagnosis of PDB [21]. An increase in ALP has been described in a large proportion of patients with metabolically active PDB and is associated with the extent of skeletal involvement [22]. Other markers of bone formation, such as serum amino-terminal peptide of procollagen type 1 (PINP) or bone-specific ALP (BALP), as well as markers of bone resorption such as serum N-terminal telopeptide (CTx) and urinary type 1

collagen (uNTX), have demonstrated better diagnostic sensitivity than ALP [23]. Elevations in these markers are characteristic but not specific for PDB, therefore they cannot be used in isolation for the diagnosis of PDB. In the absence of specific symptoms, an increase in serum alkaline phosphatase that is not associated with another pathology, such as liver disease, should lead to a search for pagetic lesions of the skeleton.

Traditional radiography of the affected bone demonstrates osteolytic and osteosclerotic areas (radiopaque and radiolucent respectively).

Bone scintigraphy is useful in assessing the skeletal involvement of the disease and is essential for identifying all affected areas.

A technetium-labeled bisphosphonate (99Tc) is used as a contrast agent, which selectively binds to bone regions with significant osteoblast activity or inflamed soft tissue.

If a single radiological lesion is characterized by osteodensifying or osteolytic changes without a clear increase in volume, which can also be observed in patients with skeletal neoplastic disease, more advanced radiological techniques such as CT or MRI are used, which can aid in the diagnosis. If doubt persists, a bone biopsy is necessary, which allows the characteristic "intertwined fiber" structure of the bone tissue to be observed.[24]

1.4. ETIOPATHOGENESIS

The pathogenetic mechanisms leading to the development of PDB remain partly unknown and likely involve the involvement of genetic and environmental factors [5] [25]. Another hypothesis is that the genetic variant of the disease represents a group of patients, while the others have a form of PDB that arises from exposure to one or more environmental triggers. Several studies suggest that the pathogenesis of PDB may be primarily linked to viral exposure of osteoclasts and their precursors. This hypothesis was advanced following the identification of viral inclusions (i.e Paramyxovirus,

measles virus and canine distemper virus) in the nuclei and, less commonly, in the cytoplasm of osteoclasts from affected patients [26].

The viral hypothesis of the disease is consistent with epidemiological observations indicating an increased prevalence of PDB in rural areas and particularly in subjects who report contact with dogs and/or other animal species, thus suggesting the involvement of zoonotic infections. [26][27].

In patients with PDB, a positive family history of the disease has been found in 10-40% of cases [28][29], although the presence of undiagnosed and asymptomatic cases makes it impossible to establish the true prevalence of familial disease.

It has been estimated that a first-degree relative of a patient with PDB has a cumulative risk of developing PDB of 9%, compared to 2% for individuals with unaffected relatives [30].

Furthermore, genetic analysis shows that at least a third of patients show a clear autosomal dominant inheritance pattern, in which case the risk of developing PDB for first-degree relatives increases to 50% (penetrance is estimated at 80%).

The main mutations in PDB have been found in the SQSTM1, ZNF687, PFN1, VCP, and TNFRSF11A genes [31].

Sequestosome 1 mutation (SQSTM1)

Mutations in Sequestosome 1 (SQSTM1) are found in approximately 40% of patients with PDB. Protein p62 plays a fundamental role in the genesis and development of PDB, as under homeopathic conditions it negatively regulates osteoclast differentiation stimulated by M-CSF and RANKL, allowing for a balance between bone formation and resorption. When p62 is mutated or eliminated, this regulation is lost with an increase of the number and size of osteoclasts, leading to osteolytic lesions. Consequently, osteoblast responses result in highly disorganized apposition to the lesions. Furthermore, with the loss of p62, osteoblasts increase the secretion of RANKL, which, by activating the NF-kB signaling cascade, further promotes osteoclast formation [32.].

The most common SQSTM1 mutation is the proline-to-leucine amino acid substitution at codon 392 (P392L) in exon 8. The majority of these mutations affect the terminal portion of the gene, specifically the ubiquitin-binding domain (UBA domain) of the protein. Patients with SQSTM1 mutations generally have a more aggressive form of the disease than patients without the mutation, with an earlier onset and a greater tendency to polyostotic forms [33] [34].

In an attempt to reconcile the viral or genetic hypothesis, in the 2011 study by Kurihara et al.[35], it was observed that, in mice, the SQSTM1 P392L mutation was primarily able to increase the sensitivity of osteoclast precursors to RANKL.

Overall, these experimental data suggest that at least some SQSTM1 mutations, such as P392L, may act as a predisposing factor (i.e., increasing sensitivity to RANKL and thus the osteoclastogenic capacity of mutation carriers), but may not be sufficient to induce PDB in most cases, and that additional factors (genetic or environmental) may be required [36.]. This could also explain the decreased penetrance or disease severity described in PDB families with the SQSTM1 mutation, as well as the greater disease burden observed in SQSTM1-positive patients who report contact with animals and/or who live in rural areas [33][37], indicating decreased exposure to a possible viral trigger in recent decades. To date, 17 mutations in the SQSTM1 gene have been identified in Italy, with a greater distribution in the Southern Italian region, especially in Campania. The presence of these mutations (such as the Y383X mutation), described almost exclusively in subjects of Campanian origin, could explain the greater severity of the disease and its early onset in patients from this region.

It remains to be clarified exactly how these changes lead to accelerated osteoclast activity, confirming the existence of defects in RANKL-induced osteoclastogenesis and the importance of p62 [31].

Profilline 1 gene mutation (PFN1)

Another important mutation recently described in PDB is profilline 1 gene (PFN1) mutation characterized by a 4-nucleotide deletion that lead a frameshift alteration. This alteration was found in two Italian pedigrees and two Chinese Families with

characteristic of early onset form of PDB [38][39]. The PFN1 gene encodes for profilin 1, a highly conserved and ubiquitously expressed protein among vertebrates, and member of the profilin family of small actin-binding proteins involved in the turnover and restructuring of the actin cytoskeleton [40][41]), that might be relevant for osteoblast biology [42][43]. In addition, profilin 1 was shown to suppress NFκB activity in the regulation of osteoclast differentiation and to prevent the degradation of the phosphatase PTEN, a known negative modulator of osteoclast differentiation [44]. Of interest, previous experimental evidences already underlined the relevance of profilin 1 for bone biology, since osteoclast specific deletion of PFN1 increased cell motility, podosome formation, size, and bone resorptive activity of osteoclasts in vitro and caused several bone abnormalities in vivo, including dwarfism, reduced trabecular bone mass and osteolytic lesions [45]. Moreover, as for other PDB genes (e.g., SQSTM1 or VCP), PFN1 mutations were previously described in patients with familial ALS [46] . After the discovery of PFN1 mutations in PDB, a transgenic mice model with the truncating D107Rfs*3 mutation of PFN1 gene was recently established [47]. While the homozygous mutation was embryonically lethal (likewise in the PFN1 KO mice), heterozygous transgenic mice were smaller in size, and showed accelerated osteoclast differentiation, with deformed craniofacial bones and the presence of focal PDB-like lesions [47]. Reduced profilin 1 expression was also demonstrated in femur sections from transgenic mice using immunohistochemistry staining. Taking all together these experimental observations suggest that the PFN1 mutations associated with PDB might confer a loss of function in profilin 1 activity and cause PDB-like features in

the osteoclasts, likely due to enhanced cell motility, increased Nf-kB activity, and actin ring formation.

Studies in humans have shown a particular phenotype characterized by symmetrical and extensive skeletal involvement (in particular skull), the presence of severe, early onset, osteoarthritis at the spine and major joints (occurring between 25 and 50 years) and an increased prevalence of fractures or neoplastic degeneration in either osteosarcoma (described in 21% of Italian patients) or GCT (described in 31% of Chinese cases). Moreover, reduced response to aminobisphosphonates, including

zoledronic acid, requiring multiple infusions to control bone pain and achieve biochemical remission over a long term was described in most of these cases [39][47]. All etiopathogenetic mechanism of PDB are resumed in **Figure 1.**

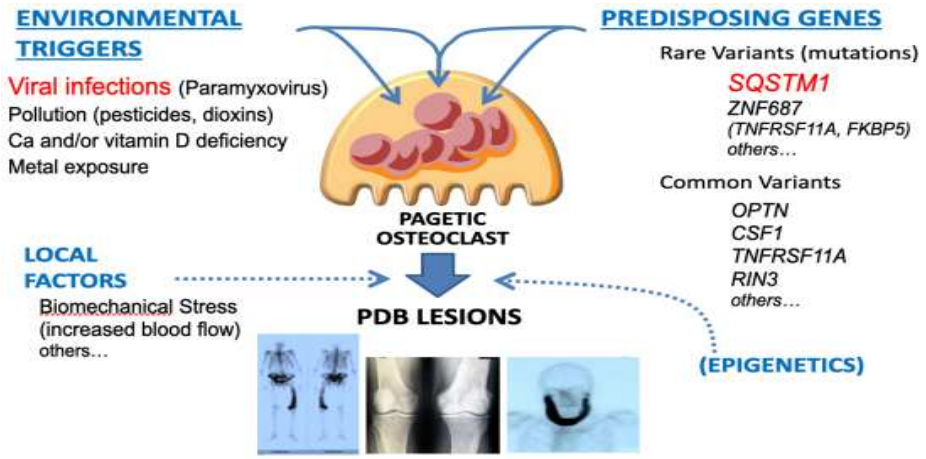


Figure 1. Schematic overview of the main environmental, genetic, local, and epigenetic factors contributing to Paget's disease of bone lesions. The diagram illustrates how viral infections, pollutants, calcium/vitamin D deficiency, metal exposure, genetic predisposition (including rare and common variants), biomechanical stress, and epigenetic mechanisms act on pagetic osteoclasts, leading to the typical radiological features of the disease.

1.5. CARDIAC INVOLVEMENT IN PAGET DISEASE OF BONE

While the primary manifestation of PDB is skeletal, it is crucial to emphasize that cardiac involvement represents a clinically relevant and often underappreciated aspect of this disease**.** In particular, cardiac manifestations can determine significant functional compromise, thereby justifying the importance of comprehensive echocardiographic evaluation in this patient population.

Studies dating back to the 1950s seem to confirm a link between PDB and cardiovascular disease, showing that increased bone vascularization causes heart failure, an increased incidence of atherosclerosis, and hypertension in affected patients. Another possible alteration demonstrated was chest deformity could compromise cardiorespiratory function [48] [49].

Cardiomegaly or ventricular hypertrophy was present in at least half of patients with PDB.[48]. For this reason, it was retained that congestive heart failure was a useful parameter indicating antiresorptive therapy [50]. Only a few studies have analyzed

cardiac complications in patients with PDB using standard echocardiographic examinations, and only two of them had a case-control design. [51],[52]] Evidence of major cardiac complications was shown in patients with a polyostotic involvement. [51 In particular, cardiomegaly, increased left ventricular diastolic dimension and increased left ventricular mass indicative of ventricular hypertrophy were found in polyostotic compared to monostotic patients. Indeed, patients with more extensive skeletal involvement presented signs of depressed myocardial contractility, increased left ventricular volumes in diastole and systole and improvement of stroke volume (SV).[51]. In a case-control study, PDB patients presented a reduction in peripheral vascular resistance with an increase in stroke volume and consequent increase in cardiac output, in particular in polyostotic involvement. [52]. Taking into account this data, an increase in cardiac output reflects in an "high flow" heart failure and subsequent increase in myocardial stress with a greater energy and oxygen consumption. Continuous stress status leads to a following pump failure [53]

It was also observed that in early phases of PDB there's a reduction in peripheral vascular resistance. The persistence of this status may lead progressively to increased cardiac output, which is mainly influenced by the degree of turnover impairment and the age of the individual.

Extraosseous calcification was another alteration common in Patients with PDB, in particular at level of the large vessels (especially in the aorta and the iliac and femoral arteries). In a study of 42 patients, 52.4% showed arteriosclerotic calcifications compared to 30.6% of controls. The length and thickness of the calcifications were greater in patients with PDB [54].

Other scientific evidence documented the presence of cardiac calcifications in patients with PDB, particularly calcium deposits both in the interventricular septum, with consequent conduction abnormalities, and in the valvular region [48].

In recent decades, clinical and experimental studies have indicated a common etiopathogenetic pattern between metabolic bone disorders and cardiovascular disease, providing evidence for a bone-cardiovascular axis [55][56] [57][58]. Possible genetic implications in PDB will be discussed below

CHAPTER 2: OBJECTIVES OF THE STUDY

This project was designed to provide an integrated characterization of Paget's Disease of Bone combining clinical, epidemiological, and molecular perspectives. To achieve this goal, the study was structured into two main tasks, each addressing complementary aspects of the disease. TASK 1 focused on the clinical and epidemiological features of PDB within the Italian Registry of Paget's Disease of Bone, one of the largest national cohorts of affected patients. This task aimed to define the spectrum of skeletal involvement, assess the prevalence of major complications, and explore possible associations between PDB and cardiovascular or metabolic comorbidities by comparison with a population-based control group from the "Siena Osteoporosis Study" (SiOP). TASK 2 investigated the cardiac phenotype associated with PDB, with particular emphasis on the influence of genetic mutations such as SQSTM1 and PFN1 on cardiac structure and function. Through echocardiographic analyses, this part of the study demonstrated that different genotypes may be linked to distinct remodeling patterns, suggesting a genotype-specific cardiac adaptation to the hemodynamic changes induced by increased bone turnover.

Together, these two research components provide a comprehensive picture of PDB as a systemic disorder, bridging the gap between its skeletal manifestations, cardiovascular implications, and underlying molecular mechanisms.

2.1. TASK 1: Aims and Rationale

Paget's disease of bone (PDB) is a chronic disorder of bone remodeling characterized by abnormal osteoclastic activation, accelerated bone turnover, and structurally disorganized bone formation. The clinical expression of the disease is remarkably heterogeneous, ranging from asymptomatic monostotic lesions to severe, deforming, and polyostotic forms. Over the past decades, several epidemiological studies have

documented a striking decline in both the prevalence and severity of PDB across generations, suggesting that the interplay between genetic background and changing environmental factors may deeply influence disease expression.

The Italian Registry of PDB, one of the largest national cohorts of patients with this condition, offers an invaluable opportunity to explore these variations in phenotype and disease progression. Through the systematic collection of clinical, biochemical, and genetic data, the registry provides the basis for an integrated evaluation of how molecular alterations, such as Sequestosome 1 (SQSTM1) or Zinc-finger nucleases 687 (ZNF687) mutations, translate into specific clinical patterns and outcomes.

The chronic hypervascularity of active pagetic bone, the persistent inflammatory milieu, and the altered mineral metabolism observed in these patients could, in theory, exert systemic effects on cardiovascular function. However, the extent and clinical relevance of these associations remain uncertain.

To clarify these aspects, this study compared patients enrolled in the Italian Registry of PDB with a well-characterized control population derived from the Siena Osteoporosis Study (SiOP). This large cohort of elderly Italian men and predominantly postmenopausal women serve as a reliable reference for the evaluation of bone health, metabolic parameters, and comorbidity profiles in the general population.

The main objective of this work was therefore to provide a comprehensive characterization of Paget's disease in the Italian population, assessing its clinical spectrum, genetic background, and systemic involvement. By integrating epidemiological, biochemical, and genetic data, this thesis aimed to elucidate whether PDB, beyond its skeletal expression, may represent a condition with broader systemic implications, particularly in relation to cardiovascular health and metabolic regulation.

2.2. TASK 2: Aims and Rationale

A) CARDIAC INVOLVEMENT IN PDB: FROM THE PAST TO THE FUTURE

The primary objective of this Task is to comprehensively and quantitatively assess the cardiac involvement in patients diagnosed with Paget's Disease of Bone. In particular:

- Detailed echocardiographic characterization of cardiac anatomy and function in PDB, focusing on parameters such as left ventricular dimensions, wall thickness, ejection fraction, valvular function, and pulmonary artery pressures.
- Identification and analysis of any abnormalities in cardiac structure or performance that may correlate with disease severity or duration. Particularly, we focus on diastolic function and cardiac remodeling.
- Exploration of different genetic phenotypes (particularly SQSTM1 and PFN1) involved in the pathophysiology of cardiac manifestations in PDB, through correlation of clinical findings with genetic testing results.

All objectives have been designed with measurable endpoints to ensure robust and reproducible evaluation of hypotheses. This approach aims to provide a clearer understanding of the cardiac phenotype in PDB patients and to inform future clinical management strategies.

Actually, there is few data regarding cardiac involvement in PDB patients.
In the past, several studies have been conducted to evaluate the cardiac effects of PDB, concluding that a long-term disease leads to 'high-output heart failure'. [48][50][51]. At the base of this condition, the most involved pathophysiological mechanisms were the increased vascularization of pagetic bone and the rise of cardiac pump work. An increase in bone turnover and the consequent blood demand at the PDB-bone would lead to an increase in vascularization. As demonstrated by bone biopsies, vessels appear hyperplastic, vascular trees present numerous shunts and the presence of inflammation (with an increase in cytokines at that level) causes hyperemia [48].

Increasing bone metabolism indirectly induced a major blood request to peripheral capillaries that appear dilated with consequent reduction in peripheral vascular resistance. The greater recruitment of blood in the pagetic sites together with the high vascularization are both responsible for a condition of cardiac stress. In fact, an increase in stroke volume (SV) subsequently induced to an increase in cardiac output (CO) that is responsible for major pump work [52]. This situation configures a pattern of cardiac insufficiency that is called "High Flow Heart failure" that is characterized by a strong cardiac stress with the incapacity of the Heart to satisfy the energy/blood request of periphery. The long-term consequence is a pump failure with worsening cardiac function that leads to congestive Heart Failure. Moreover, other PDB-related conditions such as aortic and valvular calcification could contribute to the exacerbation of cardiac involvement. **Figure 2**

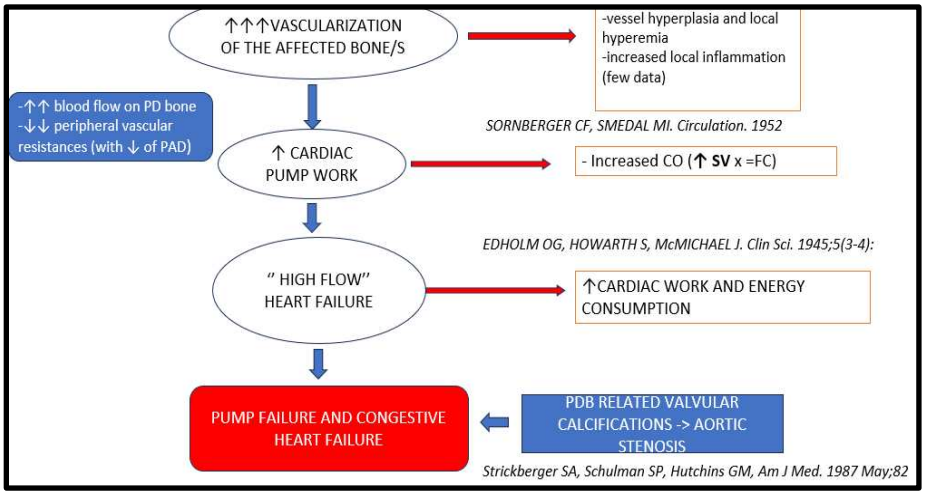


Figure 2 : Schematic representation of pathophysiology in cardiac involvement of PDB, according to past studies

Although previous studies have provided valuable insights into the pathophysiological processes underlying cardiac involvement in Paget's disease, their limitations must be acknowledged. Most of the patients included were in advanced stages of the disease, which is associated with a higher prevalence of long-standing skeletal and extraskeletal complications. Furthermore, limited knowledge regarding therapy or incomplete treatment—most patients were treated with calcitonin—hindered efforts to prevent the progression of PDB. The introduction of bisphosphonate therapy, particularly amino-

bisphosphonates, has dramatically reduced the incidence of advanced cases, except for certain aggressive genetic forms. It is highly probable that reducing bone turnover may lead to decreased peripheral vascularization and, consequently, reduced cardiac stress.Another limitation of these studies is the small sample size. In addition, the use of outdated or suboptimal tools for cardiac assessment (e.g., chest X-ray) or instruments constrained by the technology available before 2000 may have produced less accurate results compared to the advanced diagnostic tools currently available. Advances in medicine now allow for detailed investigation of cardiac function in Paget's disease, and early identification of cases enables observation of these effects in the initial stages and their progression over time. Moreover, targeted diagnostic approaches for both PDB and cardiovascular disease (CVD) have been refined, improving our understanding of the disease. Considering these points, our evaluation is based on the current knowledge of PDB and cardiac assessment, and our rationale is illustrated in the following **Figure 3**

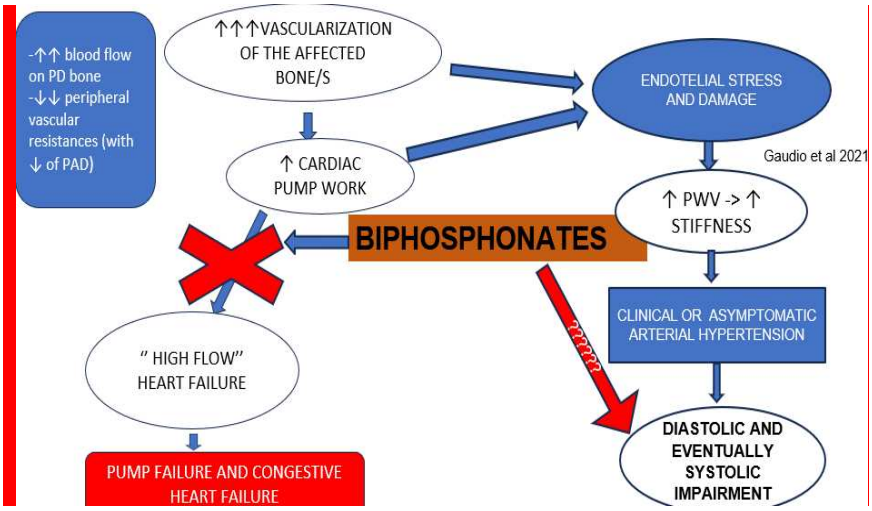


Figure 3: Our pathophysiological point of view in cardiac PDB involvement

We maintain that the fundamental mechanisms initiating the disease are consistent with those previously demonstrated[48]. Initial damage is likely related to increased vascularization of the affected bone. This physiological change not only increases the

workload of the cardiac pump but also promotes endothelial damage, which in turn contributes to increased vascular stiffness . At the cardiac level, these alterations may lead to remodeling of the cardiac chambers, the development of arterial hypertension, elevated ventricular filling pressures, and atrial enlargement.

Bisphosphonates, the most common drugs used in PDB, have demonstrated potential indirect vascular benefits. Their structural similarity to inorganic pyrophosphate enables them to inhibit vascular calcification by binding to hydroxyapatite, thereby preventing calcium crystal deposition in the vascular wall [54] Additionally, they may modulate inflammation and mineral metabolism, influencing cytokine activity and reducing systemic inflammatory burden, which could contribute to improve vascular health [55] However, despite these promising findings, no direct cardioprotective effects have been conclusively demonstrated. The observed associations between bisphosphonate use and reduced vascular calcification or improved arterial compliance are largely indirect and may depend on underlying bone–vascular cross-talk mechanisms. Further studies are necessary to evidence this link. Our hypothesis suggests that early use of bisphosphonate (in particular, Aminobisphosphonates) can block or decelerate PDB-related cardiac dysfunction, avoiding the evolution in Heart Failure (HF).

From March 2019 to October 2022, we conducted a study in collaboration with the University Federico II (Naples, Campania, Italy), enrolling 69 patients with PDB—both monostotic and polyostotic—and 115 matched controls. Patients with pre-existing structural heart disease or cardiac abnormalities were excluded from the analysis. Standard echocardiography revealed that PDB patients exhibited significantly higher interventricular septal diameter (IVS), pulsed-wave tissue Doppler parameters, relative wall thickness (RWT), left ventricular mass index (LVMI), E/e' ratio, right ventricular transverse diameter, and systemic vascular resistance compared to controls. Contrary to previous observations, in our cohort, left ventricular ejection fraction (LVEF), stroke volume (SV), and cardiac output were significantly lower in PDB patients compared to controls ($p < 0.05$ for all). However, regarding systolic function, it should be noted that LVEF values in both groups remained within the range of preserved ejection fraction (pEF) and are therefore not clinically significant. A more

sensitive assessment of systolic performance was provided by advanced echocardiographic evaluation using global longitudinal strain, which demonstrated reduced systolic efficiency in PDB patients. Speckle-tracking echocardiography further confirmed lower global work efficiency compared to controls ($p < 0.05$). Consistent with prior studies, PDB patients also showed a higher prevalence of sclerosis and/or calcification of the aortic and mitral valves compared to controls ($p < 0.05$) [56]. The findings of this study suggest a potential subclinical impairment of systolic function, with possible diastolic dysfunction. In the subsequent sections, we focus in detail on diastolic function, cardiac remodeling, and additional parameters of cardiac involvement in PDB, while also considering the effects of bisphosphonate therapy duration and the relationship between therapy initiation and cardiac alterations.

B) FOCUS ON ROLE OF SQSTM1/P392 L- MUTATION IN CARDIAC FUNCTION: CAN THIS MUTATION AFFECT HUMAN HEART?

The relationship between oxidative stress and cardiac damage in the PDB patients affected by SQSTM1 mutations is an area of growing interest. Direct evidence linking these mutations to cardiac toxicity is limited. Several lines of indirect evidence suggest that the oxidative stress associated with defective p62 function could contribute to cardiac damage (studies in mice and vitro/models) [57]. Particularly, studies evidenced the impact on endothelial damage and cardiac stress, hypertrophy and other alterations in cardiac mice KO for different locus of SQSTM1 transcription for p62. Particularly it was shown that cardiomyocyte-specific p62 knockout mice develop cardiac hypertrophy and fibrosis with age, indicating a protective role of p62 in maintaining cardiac function during aging [58]. Another study evidenced that Endothelial p62 knockout mice show multi-organ fibrosis and cardiac dysfunction, highlighting the importance of p62 in endothelial cells for cardiovascular health.[59]. Li et al say that cardiomyocytes are highly susceptible to oxidative damage due to their elevated metabolic activity and mitochondrial density. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant

defenses, can lead to mitochondrial dysfunction, apoptosis, fibrosis, and inflammation—all of which are hallmarks of cardiac injury. In models where autophagy is impaired or p62 function is compromised, increased ROS levels have been linked to cardiac hypertrophy, cardiomyopathy, and reduced contractile function. [60]. In our knowledge, no data in humans were found. In patients with P392L deletion, the antioxidant function of p62/SQSTM1 is not directly impaired. However, these mutations can lead to an indirect compromise of this function due to the disruption of other cellular processes. One of p62's roles is to activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a key regulator of antioxidant responses. This activation occurs through the sequestration of Kelch-like ECH-associated protein 1 (Keap1), which normally represses Nrf2 activity. By binding to Keap1, p62 prevents its interaction with Nrf2, thereby promoting the expression of antioxidant genes [61] In PDB, most disease-associated SQSTM1 mutations affect the C-terminal ubiquitin-associated (UBA) domain of p62[31]. These mutations impair p62's ability to bind ubiquitinated proteins, leading to defective autophagy and the accumulation of damaged proteins and organelles, but the normal activation of Nrf2 gene does not directly interrupt antioxidant function.[62] However, the increased oxidative stress resulting from impaired autophagy can overwhelm the antioxidant defenses, leading to cellular dysfunction. Although this hypothesis appears rational, recent studies (Milan E, Cenci S. et al. University" Vita-Salute S. Raffaele", Milan, unpublished) on P394L mutant mice (the murine analogue of the human P392L mutation) have shown increased muscle strength and performance. This data suggests that the presence of a SQSTM1 mutation may confer metabolic and functional advantages in patients with mutation. The proposed explanation suggests that the truncated p62 protein, unable to perform its normal autophagic functions, may instead enhance mitochondrial energy stimulation. This shift could lead to elevated energy production and consequently improved muscular performance. It is further hypothesized that this mitochondrial energetic effect is mediated by a protein that, when overactivated, amplifies this process. Ongoing studies aim to elucidate the molecular mechanisms underlying these observations. Based on this hypothesis, we conducted a collaborative study with University "Vita-Salute San Raffaele" (Milan, Italy) to investigate whether similar

effects are observed in human Paget's disease of bone (PDB) patients carrying the P392L mutation. We analyzed a cohort of 450 PDB patients from the Senese cohort of the Italian Paget Registry (392 wild-type and 58 with SQSTM1/P392L mutations). Morbidity, treatment history, and mortality rates over a 20-year follow-up period were assessed and compared with 1120 control subjects enrolled in the "Siena Osteoporosis Study ", a registry of healthy individuals evaluated for phosphor-calcic metabolism (a brief description of the study will be given later, in detail). Mortality was significantly higher in PDB patients compared with controls (24% vs. 18%, $p < 0.005$). Although not statistically significant, mortality was also higher in SQSTM1-mutated patients compared with wild-type individuals. The mean age at death was significantly greater in P392L+ patients compared with controls (82–96 vs. 58–94 years, $p < 0.01$), and similarly elevated in wild-type PDB patients versus controls (61–97 vs. 58–94 years, $p < 0.01$). Despite a trend toward increased age at death in P392L+ patients compared to wild-type (82–96 vs. 61–97 years), this difference was not statistically significant ($p = 0.15$). Muscular fitness and performance were assessed using the Short Physical Performance Battery (SPPB), handgrip strength, Instrumental Activities of Daily Living (IADL), and Activities of Daily Living (ADL) tests. Among patients aged approximately 70 years, those carrying the SQSTM1 mutation exhibited significantly higher muscular and neuromotor performance, particularly in the SPPB test (completed by 65% of wild-type vs. only 25% of mutated patients, $p < 0.05$), with comparable trends across other tests. In relation to the contrasting evidence reported in the literature, this study aims, for the first time to our knowledge, to further evaluate the effects of the SQSTM1 mutation on cardiac function through echocardiographic assessment, in order to determine whether specific cardiac alterations are associated with these genetic forms compared with healthy and wild-type PDB patients. The results are resumed in the following **Figure 4 and Figure 5**

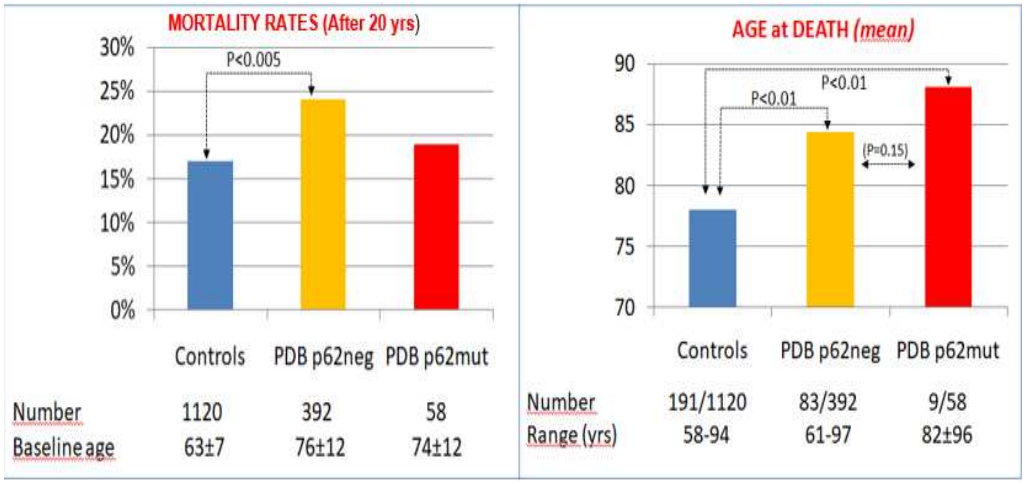


Figure 4. Mortality rate and age of death comparison between controls (Controls) , wild type patients (PDB p62 neg) and SQSTM1/P392L patients (PDB p52 mut)

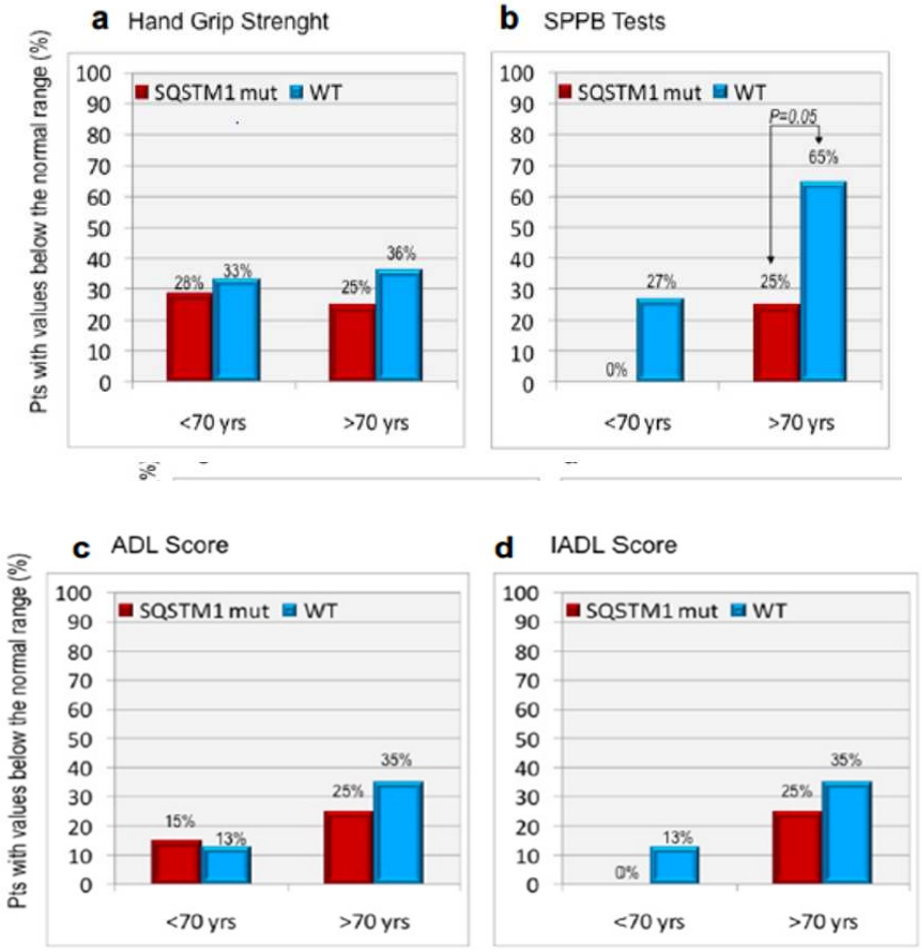


Figure 5 Different performance tests, indicated respectively as (a) SPPB, (b) handgrip strength, (c) ADL, and (d) IADL, demonstrated that among patients older than 70 years, subthreshold performance values were more frequently observed in wild-type individuals than in those carrying the SQSTM1 mutation. However, statistical significance was reached only for the SPPB test.

C) PFN 1 AND CARDIAC FUNCTION: DOES THIS RELATIONSHIP EXISTS?

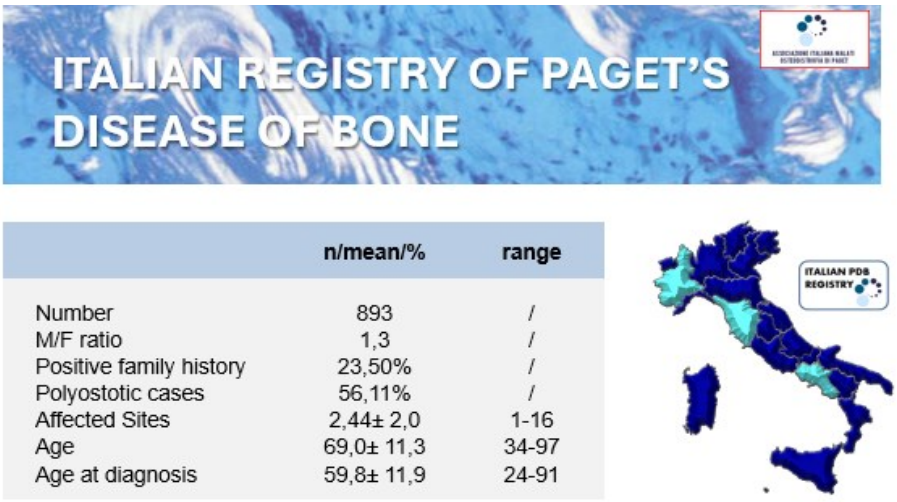
While Profilin 1 (PFN1) mutations are primarily linked to skeletal abnormalities in PDB, the actin cytoskeleton's role in cardiomyocytes suggests that PFN1 could influence cardiac function. Profilin-1's involvement in actin dynamics is essential for maintaining sarcomere structure and function [45]. Disruptions in actin polymerization can lead to impaired contractility and altered calcium handling in cardiomyocytes [63]. However, the absence of clinical evidence linking PFN1 mutations to cardiac damage suggests that any such effects, if they exist, are either subtle or clinically insignificant. Further research is needed to explore the potential cardiac implications of PFN1 mutations in greater detail.

CHAPTER 3: MATERIALS AND METHODS

3.1. TASK 1: Clinical, Epidemiological, and Genetic Characterization of Paget's Disease of Bone: Insights from the Italian PDB Registry

3.1.1. STUDY POPULATIONS

The study population, consisted of 893 patients (male/female ratio = 1.3) enrolled in the Italian Registry of Paget's Disease of Bone, a nationwide multicenter database including participants from specialized metabolic bone units throughout Italy.
A positive family history of PDB was reported in 23.5% of patients. The disease was polyostotic in 56.1% of cases, whereas the remaining patients showed monostotic involvement. The mean number of affected skeletal sites was 2.44 ± 2.0, ranging from 1 to 16. The mean age of the cohort at the time of the last clinical evaluation was 69.0 ± 11.3 years (range 34–97), while the mean age at diagnosis was 59.8 ± 11.9 years (range 24–91). Patients were recruited consecutively, and diagnostic confirmation was based on standard clinical, biochemical, and imaging criteria. Geographic distribution of cases across Italian regions is shown in the accompanying map, illustrating the national coverage of the registry and the predominance of cases from Northern and Central Italy. **Figure 6**



	n/mean/%	range
Number	893	/
M/F ratio	1,3	/
Positive family history	23,50%	/
Polyostotic cases	56,11%	/
Affected Sites	2,44± 2,0	1-16
Age	69,0± 11,3	34-97
Age at diagnosis	59,8± 11,9	24-91

Figure 6. Italian Registry of PDB: Baseline Characteristics Demographic and clinical data of 893 Italian PDB patients. M/F ratio 1.3; 23.5% familial cases; 56.1% polyostotic; mean affected sites 2.44 ± 2.0 (range 1–16); mean age 69 ± 11.3 yrs; mean age at diagnosis 59.8 ± 11.9 yrs.

The Siena Osteoporosis Study (SiOP) is an ongoing population-based project initiated in 2003, designed to evaluate the prevalence of osteoporosis and bone fragility in elderly Italian men and postmenopausal women. Through the collaboration of general practitioners in the Tuscany region, a random sample of 1149 subjects aged 50–80 years (174 men, mean age 65.9 ± 6 years; 975 women, 62.5 ± 6 years) was recruited between 2004 and 2009.

3.1.2. CLINICAL DATA COLLECTION

Comprehensive clinical histories were obtained, including disease duration, treatment regimens, and comorbidities. Data on prior fractures, bone pain, and biochemical markers of bone turnover were also recorded.

Anthropometric data (height and weight) were collected using standardized instruments, and information on sociodemographic status, lifestyle factors, and comorbidities was recorded through validated questionnaires.

Cardiovascular risk factors (hypercholesterolemia, diabetes mellitus, hypertension, stroke, atrial fibrillation, myocardial infarction, and heart failure) were systematically documented.

The study was conducted in accordance with the Declaration of Helsinki, with ethical approval (Protocol 961.03, 29 October 2013) and written informed consent from all participants.

3.1.3. GENETIC ANALYSIS

Peripheral blood samples were collected for DNA extraction. Genetic screening focused on known PDB-associated mutations, such as those in the SQSTM1 and ZNF687 genes, using PCR-based methods and sequencing for mutation detection.

3.1.4. BIOCHEMICAL ASSAY

Serum levels of alkaline phosphatase, calcium, phosphate, and other markers indicative of bone metabolism were measured using standardized laboratory protocols.

3.1.5. STATISTICAL ANALYSIS

Descriptive statistics summarized clinical and genetic data. Comparative analyses between subgroups (e.g., mutation carriers vs non-carriers) were performed using Student's t-tests or Mann–Whitney U tests for continuous variables, and chi-square tests for categorical variables. Correlations were assessed with Pearson or Spearman coefficients. Statistical significance was set at $p < 0.05$.

3.2. TASK 2: Echocardiographic Evaluation of Cardiac Function in Relation to Genetic Background in PDB

3.2.1. STUDY POPULATION

The study was conducted on a cohort of 87 patients with a confirmed diagnosis of Paget's disease of bone from the Osteo-metabolic Diseases outpatient clinics pertaining

to Internal Medicine and Complexity Unit of the University Hospital of Siena (Siena, Tuscany, Italy). Adult patients (≥18 years) with a confirmed diagnosis of PDB based on clinical, radiological, or biochemical evidence, according to the latest international guidelines, were included in the analysis [21] [23]. Twelve on 86 patients were excluded from analysis despite performance of visit and echocardiography (see section. *Exclusion Criteria*). All the patients who accepted the exams writing a informed consenst, able to undergo comprehensive transthoracic echocardiographic examination , according to standardized protocols, participated in the study

The pagetic population was compared with 153 not-pagetic patients from outpatient clinics, afferent to Cardiovascular Disease Unit of University Hospital of Siena (Siena, Tuscany, Italy). The patients underwent an anamnestic evaluation including height, weight, blood pressure, heart rate, BMI, BSA, and history of hypertension. All patients in the study were given informed consent [64]. Both the population presented a high number of males respect to females (PDB Males vs Females, 72 vs 28%; Controls Males vs Females, 75 vs 25%; p=0.7)

3.2.2. LABORATORY ANALYSIS

All PDB patients were submitted to laboratory tests performed using serum samples collected in the morning after an overnight fast. Principal parameters analyzed were: Serum concentrations of calcium (Ca2+), phosphate (P), creatine (Cr), total alkaline phosphatase (ALP) and bone alkaline phosphatase (BALP), 25-OH vitamin D (25-OH-D), and parathyroid hormone (PTH). On the following **Table 1** the dosages of our pagetic population.

Parameter	Values (Mean ± SD)	Reference Range
Ca^{2+} (mg/dL)	9.33 ± 0.73	8.6–10.4
Phosphate (mg/dL)	3.33 ± 0.59	2.5–4.8
Creatinine (mg/dL)	0.93 ± 0.20	0.6–1.2
Total ALP (U/L)	140.61 ± 161.92	3–105–120
BALP (mcg/L)	30.97 ± 42.26	6–30
25-OH Vitamin D (ng/mL)	37.01 ± 15.22	>30 (sufficient)
PTH (pg/mL)	43.97 ± 25.66	7–65

Table 1. Laboratory Values in PDB Population. Laboratory biochemical parameters in patients with Paget's Disease of Bone (n=75). Values are expressed as mean ± standard deviation. Normal reference ranges are provided for comparison.

3.2.3. GENETIC ANALYSIS

All the patients were submitted to genetic analysis, conducted by collection of serum samples. All the samples were evaluated in the Genetic Unit of IRCCS Casa Sollievo della Sofferenza Hospital (San Giovanni Rotondo, Puglia, Italy). Among the PDB patients of the cohort, 24 tested positives for the SQSTM1 gene mutation and 3 presented PFN1 mutation.

3.2.4. EXCLUSION CRITERIA

To rule out any interference from other causes of cardiac impairment in PDB patients. The patients presenting the following criteria were excluded: history of cardiac arterial diseases (CAD); acute myocardial infarction (AMI); Uncontrolled arterial hypertension; persistent or chronic atrial fibrillation (AF) stroke; patients affected by heart failure (HF) both with reduced or preserved ejection fraction (HFrEF and HFpEF respectively), in particular patients in NYHA class (III-IV) ;severe valvopathy, diabetes mellitus (DM); dyslipidemia, chronic kidney disease (CKD, considering an estimated glomerular filtration rate calculated using the EPI-CKD formula of<60 mL/min/1.73 m2 ; active or previous history of metastatic cancer and poor-quality echocardiograms. In our analysis patients excluded for these causes were 12 (4 patients

affected by AF, 2 with CAD, 2 with CKD and other 3 for multiple diseases); Autoimmune or systemic inflammatory diseases; Chronic obstructive pulmonary disease or severe respiratory disorders.

3.2.5. ECHOCARDIOGRAPHIC PARAMETERS EVALUATION

Two-dimensional Doppler echocardiography was performed on each subject by the same physician, blinded to the patient's status.

All studies were performed using a *General Electric Vivid E9* XDclear ultrasound system combined with a 3 MHz Doppler transducer. The study was conducted with patients in the left lateral decubitus position.

We evaluated the following echocardiographic parameters: left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), Left ventricle ejection fraction (LVEF), interventricular septal thickness (IVS), end-diastolic posterior wall diameter (PWd), E to A peak velocity ratio (E/A), mean medial and septal E/e' ratio, pulmonary artery systolic pressure (PAPs), tricuspid annular plane systolic excursion (TAPSE), relative wall thickness (RWT), and left ventricular mass index (LVMI).

LV internal dimensions were measured over three cardiac cycles at end-diastole and end-systole according to updated American Society of Echocardiography guidelines [65]. M-mode measurements were made in the parasternal long-axis view. LVEDD was obtained by timing the ECG Q-wave, while LVESD was measured from maximal systolic excursion of the interventricular septum. The interventricular septum and posterior wall thickness were also measured at end-diastole.

LV volumes were calculated in the apical four-chamber view by tracing the endocardial border and LV length (distance from the mitral valve plane to apex). Volumes were indexed to body surface area (BSA) calculated by the Dubois formula [66]. Left Ventricle Ejection fraction was computed by Simpson's method, using the formula LVEF% = [(LVEDD - LVESD) × 100] / LVEDD. [66].

Pulsed-wave Doppler was used to sample mitral inflow at the mitral leaflet tips, aligned with blood flow by color Doppler. This generated a velocity profile with two peaks: E (early filling) and A (atrial contraction). The mean E/e' ratio, derived from septal and lateral mitral annulus velocities, was used to estimate left ventricular filling pressures. The E/A ratio reflects LV relaxation and filling. Left atrial volume indexed to BSA (LAVI) with a cutoff >34 ml/m² indicates atrial enlargement.
Tricuspidal regurgitation volume max (TR V max) was measured at level of tricuspidal annulus with continuous wave Doppler.

3.2.6. DIASTOLIC FUNCTION EVALUATION

For left ventricular diastolic function evaluation we consider 4 degrees above the normal level, according to international guidelines. The following parameters were considered for analysis, in line with international guidelines [67]
As reported in **Figure 7** First step considers the evaluation of Mitral inflow with analysis of the E/A ratio: a low ratio (≤0.8 with low E) typically indicates impaired relaxation, while a very high ratio (>2) reflects a restrictive filling pattern. However, mid-range values (0.8–2) are nondiagnostic by themselves, which is why supportive parameters are essential.
E/e′ (average septal and lateral) estimates LV filling pressures. Values >14 suggest elevated pressures, but must be interpreted in context (age, annular calcification, regional dysfunction can alter results). LAVI represents chronic exposure to elevated filling pressures. An enlarged LA (LAVI >34 ml/m²) strongly supports the diagnosis of chronic diastolic dysfunction.TR Vmax reflects secondary pulmonary hypertension. A value >2.8 m/s adds weight to the diagnosis of elevated LV filling pressure. Together, these three parameters provide complementary perspectives: E/e′ for current hemodynamics, LAVI for chronicity, TR Vmax for downstream consequences.
Taking into account these evaluations we obtain 4 degrees of diastolic dysfunction:

- **Grade I (impaired relaxation)**: low E/A, normal filling pressures. Common in aging, often asymptomatic.

- **Grade II (pseudonormal)**: transmitral inflow appears “normal” but additional parameters reveal elevated pressures. This grade is most challenging and requires careful integration of supportive variables.
- **Grade III–IV (restrictive filling)**: marked elevation of E/A (>2) with shortened deceleration time (DT), reflecting severely reduced compliance and high atrial pressures. Valsalva may distinguish reversible (Grade III) from fixed (Grade IV). These grades are strongly associated with symptoms and adverse outcomes.

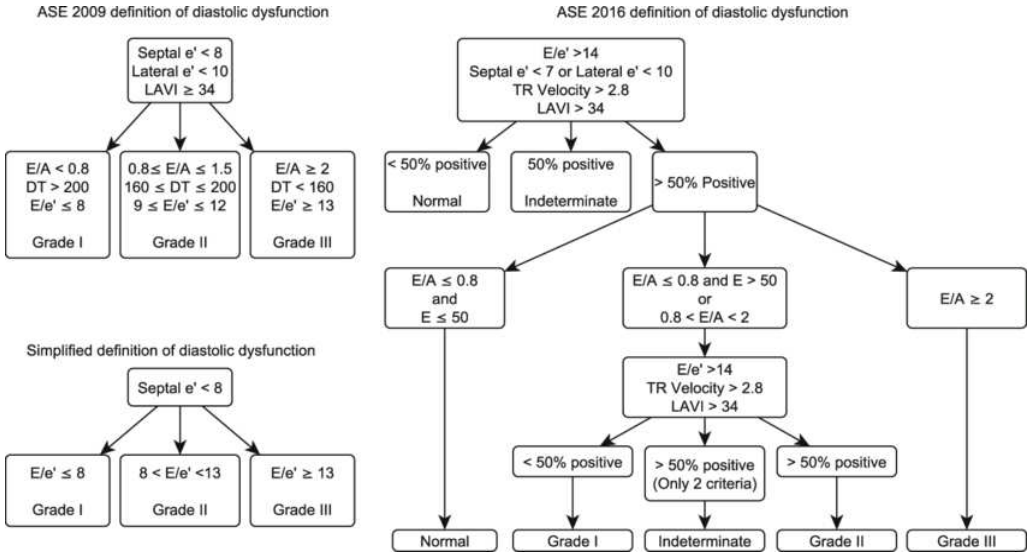


Figure 7.: The American Society of Echocardiography (ASE) 2009 and 2016 definitions of diastolic dysfunction. E, velocity of early diastolic blood flow across the mitral valve. e′, velocity of mitral annulus at early diastole. TR, tricuspid regurgitation. LAVI, left ventricular volume index. A, velocity of late (atrial) diastolic blood flow across the mitral valve. DT, deceleration time of early diastolic blood flow across the mitral valve

3.2.7. EVALUATION OF CARDIAC REMODELING

To assess the type of left ventricular hypertrophy, the relative wall thickness (RWT) was calculated according to the formula: RWT = 2PWT/DTD. The left ventricular mass (LVM) was subsequently calculated according to the Devereux formula: LVM = 0.8 * 1.04 * [DTD + SIV + (PWT)^3 - (DTD)^3] + 0.6g [68]; this was then indexed for body surface area (LVMI). Normal LVMI values are defined, according to the European Society for Cardiovascular Imaging, as 95 g/m2 for women and 115 g/m2 for men [69.].Concentric LV remodeling was defined as LVMI values <115 g/m2 in men and <95 g/m2 in women, with a RWT >0.42; left ventricular hypertrophy was

defined as LVMI >115 g/m2 in men and >95 g/m2 in women, with a RWT >0.42; eccentric hypertrophy was defined as LVMI >115 g/m2 in men and >95 g/m2 in women, with a RWT <0.42 [70]. **Figure 9**

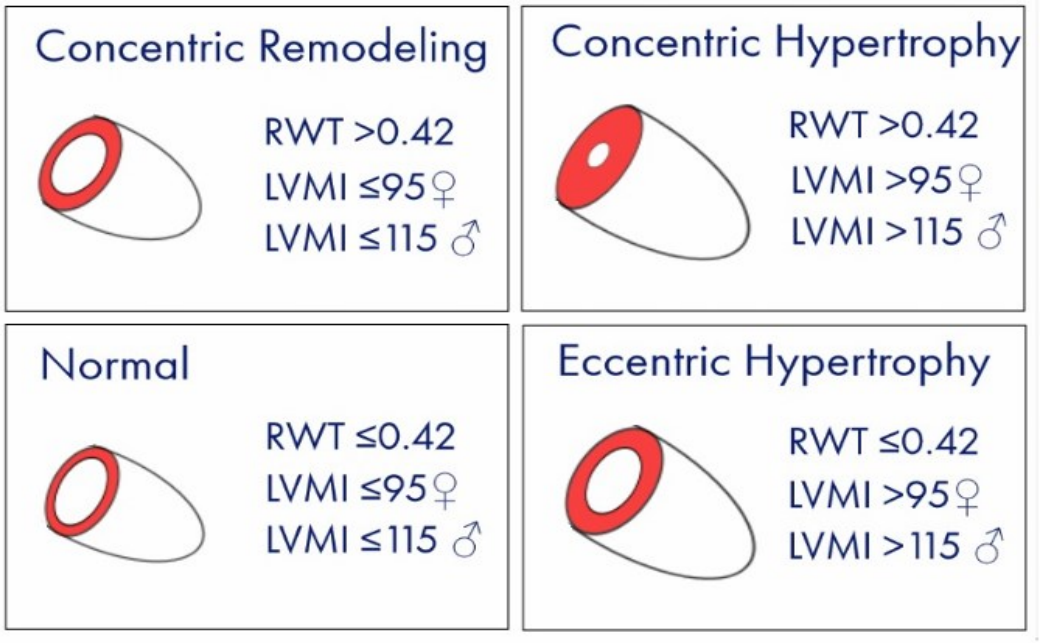


Figure 9 : Different degrees of cardiac remodeling. Illustration of the different types of left ventricular remodeling based on left ventricular mass index (LVMI) and relative wall thickness (RWT), calculated by echocardiography following standards. Concentric remodeling, concentric hypertrophy, and eccentric hypertrophy are defined by specific LVMI and RWT thresholds as shown.

3.2.8. STATISTICAL ANALYSIS

The percentage of subjects with cardiovascular disease (CVD) and cardiac valvular calcifications in the PDB patients and controls were compared using the chi-square test or Fisher's exact test. Relevant cardiac color Doppler ultrasound data, between cases and controls, were assessed using analysis of variance (ANOVA) and covariance (ANCOVA), considering age and BMI as covariates. All statistical analyses were performed with Statistical Software 6.0 for Windows (Statsoft Inc., Tulsa, U.S.A.) and SPSS software for Windows (SPSS Ltd, Chicago, IL). All statistical tests with a p value <0.05 are considered statistically significant.

CHAPTER 4: RESULTS

4.1. TASK 1 RESULTS

4 .1 .1. SKELETAL INVOLVEMENT IN THE ITALIAN REGISTRY OF PDB

A total of 893 Italian patients with confirmed Paget's Disease of Bone (PDB) were enrolled in the Italian Registry, with a male-to-female ratio of 1.3:1 and a mean age at last evaluation of 69.0 ± 11.3 years (range 34–97 years). Polyostotic involvement was observed in 56.1% (n=501) of the cohort, with the remaining 43.9% (n=392) presenting monostotic disease. The average number of affected skeletal sites per patient was 2.44 ± 2.0 (range 1–16). A positive family history of PDB was reported by 23.5% (n=210) of subjects, slightly higher in polyostotic forms than in monostotic disease.

The most frequently affected skeletal sites were the pelvis (60–70% of all cases), lumbar spine (35–40%), femur (30–35%), and skull and tibia (20–25% each). Less frequently involved regions included the humerus, thoracic spine, and sacrum (each <15%), while rare lesions in the clavicle, ribs, hands, and feet were documented in <5% of subjects. Polyostotic forms typically demonstrated multiple regional involvement with frequent combinations including pelvis-femur-vertebral involvement. Monostotic disease most commonly affected the pelvis (40% of monostotic cases) or tibia (35%) alone. All imaging findings were confirmed by independent observer assessment, with central radiological revision ensuring diagnostic accuracy. The distribution pattern of skeletal involvement aligns with previously published European epidemiological data, demonstrating clear axial and proximal appendicular predominance.

Among the 893 patients, mean age at diagnosis was 59.8 ± 11.9 years (range 24–91 years), with polyostotic patients tending toward slightly younger age at diagnosis compared to monostotic forms (59.2 ± 12.1 vs. 60.5 ± 11.6 years; p = 0.12, not

statistically significant). Positive family history was more frequently noted in polyostotic patients (26.3% vs. 19.9% in monostotic; $p < 0.01$) and in younger patient cohorts, consistent with a genetic predisposition component in disease expression. **Figure 10**

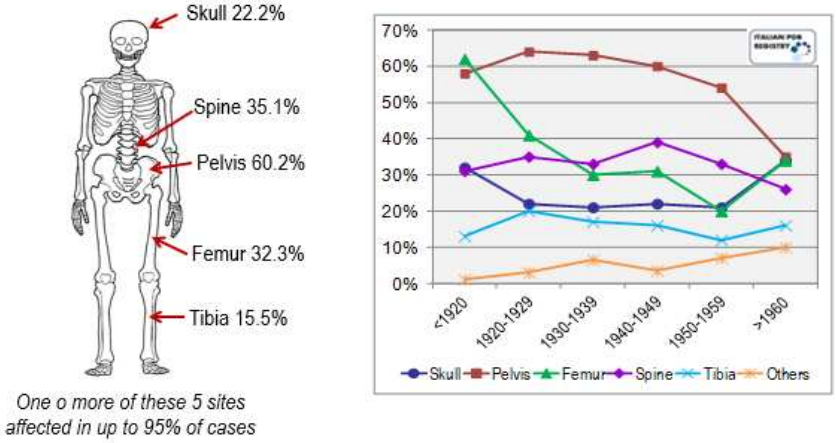


Figure 10. Skeletal Distribution of Pagetic Lesions Most common sites: pelvis (≈ 60–70%), spine (≈ 35–40%), femur (≈ 30–35%), skull/tibia (≈ 20–25%). Polyostotic cases mainly affected pelvis, femur, and vertebrae. Skeletal distribution of pagetic lesions. The most commonly affected sites include the pelvis (approximately 60–70%), spine (35–40%), and femur (30–35%), followed by skull and tibia (20–25%). Polyostotic forms mainly involve pelvis, femur, and vertebrae, while monostotic forms commonly affect the pelvis or tibia.

4.1.2. MAIN REASONS LEADING TO DIAGNOSIS OF PDB

In the present cohort, bone pain constituted the leading clinical feature prompting diagnostic evaluation for Paget's Disease of Bone (PDB), accounting for 44% of first presentations. Pain was typically described by patients as persistent, deep, and often nocturnal, leading to direct clinical referral and imaging work-up. A substantial proportion of cases (22%) were identified incidentally during radiological investigations undertaken for unrelated clinical concerns; these individuals were asymptomatic at the time of diagnosis and recognized via classic imaging findings characteristic of PDB.

Notably, isolated biochemical abnormalities contributed to early recognition in 18% of subjects, most commonly manifesting as an isolated elevation of serum alkaline phosphatase detected during routine laboratory screening. Less frequently, diagnostic suspicion arose following bone scintigraphy performed for alternate indications (5%

of cases), or the presence of pronounced skeletal deformities (3.5%) or pathological fractures (3%).

Additional diagnostic triggers included sensorineural hearing loss, nephrolithiasis, and a positive family history, each accounting for a small but clinically relevant proportion of initial assessments. The heterogeneity and subtlety of presenting features underscore the necessity of heightened clinical vigilance and targeted screening in populations at risk. Across the entirety of follow-up, bone pain and deformity remained the most prevalent and clinically meaningful symptoms, both justifying initial evaluation and reflecting ongoing disease burden.

This distribution of diagnostic pathways highlights the multifaceted nature of PDB presentation and reaffirms the role of both symptomatic and asymptomatic case-finding in optimizing early recognition and management strategies within contemporary clinical practice. **Figure 11**

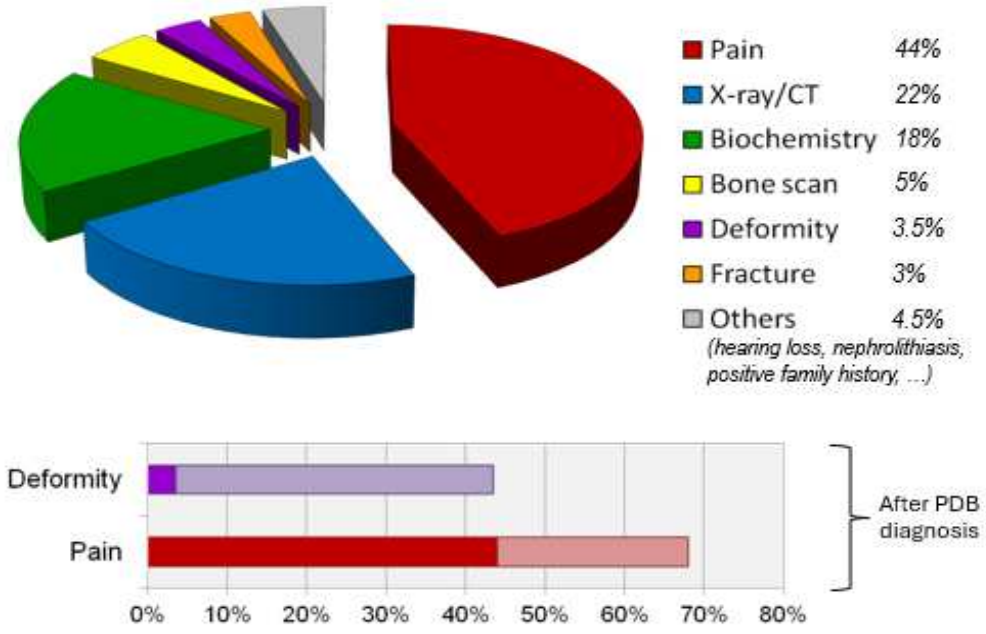


Figure 11. Main reasons leading to PDB diagnosis. Bone pain is the leading initial symptom (44%), followed by incidental radiological findings (22%) and biochemical abnormalities (18%). Less frequent causes include bone scintigraphy, skeletal deformities, and pathological fractures.

4.1.3 TREND OF DISEASE SEVERITY BASED ON DATE OF BIRTH

Analysis of the Italian registry cohort reveals a marked temporal trend in disease severity of Paget's Disease of Bone, observed across birth cohorts spanning from the early twentieth century to recent decades. Patients born prior to 1920 exhibited substantially more severe disease manifestations, as reflected by a higher mean number of affected skeletal sites (average 3–4 per subject) and a predominance of polyostotic involvement, which accounted for over 80% of cases.

This pattern underwent progressive modification in subsequent generations, with a statistically significant reduction in disease extent. In individuals born after 1960, the average number of affected sites fell to approximately 2, and the prevalence of polyostotic disease declined to 40–50%. Regional analysis further accentuated this trend, particularly among patients from Campania, where the mean number of sites per patient dropped from 5 to 2–3, and polyostotic forms decreased from greater than 90% to nearly 50%. Multivariate regression confirmed that birth cohort was independently associated with both the number of involved skeletal sites and the proportion of polyostotic forms ($p < 0.001$ for trend). These findings suggest generational attenuation of disease severity over time, likely attributable to evolving environmental exposures, improved early diagnosis, and the widespread adoption of bisphosphonate-based therapies beginning in the early 2000s. **Figure 12**

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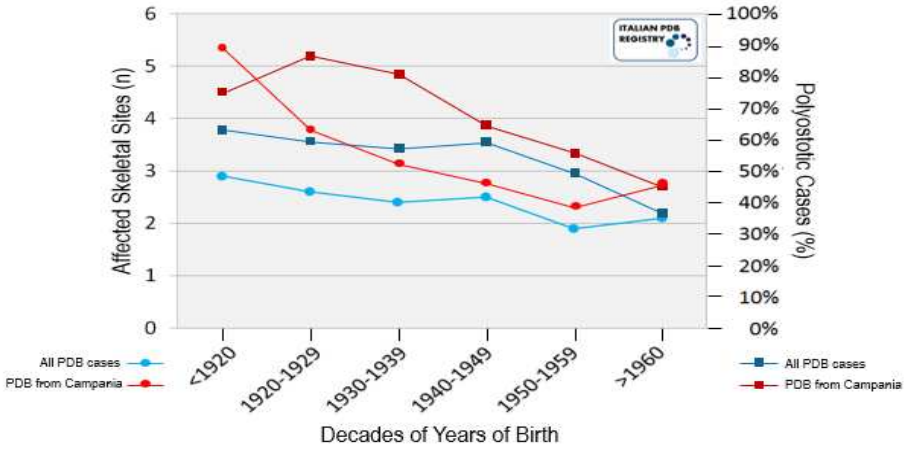

Figure 12. Trend in disease severity according to birth date. Both the average extent of osteolytic sites and proportion of polyostotic forms significantly decreased in more recent birth cohorts, reflecting epidemiological improvement.

4.1.4. MAJOR COMPLICATIONS IN ITALIAN REGISTRY

The prevalence of major disease-related complications was analyzed.

As illustrated in **Figure 13**, the most frequent complication was general osteoarthritis (OA), which was observed in 56.7% of all patients. When OA was specifically localized to Pagetic bone sites, the prevalence was 42.5%, particularly in weight-bearing bones such as the pelvis, femur, and spine. Fractures represented another major clinical burden, affecting 27.6% of patients overall; 12% of these fractures occurred directly at Pagetic sites, typically involving tibia or femur.

Hearing loss was reported in 33.3% of subjects, and its frequency was significantly higher in patients with skull involvement, reflecting the mechanical and neurological impact of cranial lesions.

Neurological complications (e.g., spinal cord compression, radiculopathy, or cranial nerve deficits) were documented in 6.5% of the cohort.

Nephrolithiasis was detected in 16%, while primary hyperparathyroidism was present in 4% of patients, often associated with biochemical evidence of hypercalcemia.

Malignant transformation was rare, with osteosarcoma occurring in 0.6% and giant cell tumor in 0.7% of cases. Interestingly, none of these malignancies were observed in patients carrying SQSTM1 gene mutations, and the prevalence of hyperparathyroidism was also lower in this subgroup.

Overall, these data demonstrate that osteoarthritis, fractures, and hearing loss are the predominant clinical complications of PDB in Italy. The figure also highlights the increased risk of OA in weight-bearing bones and of neurological impairment in skull-involved disease.

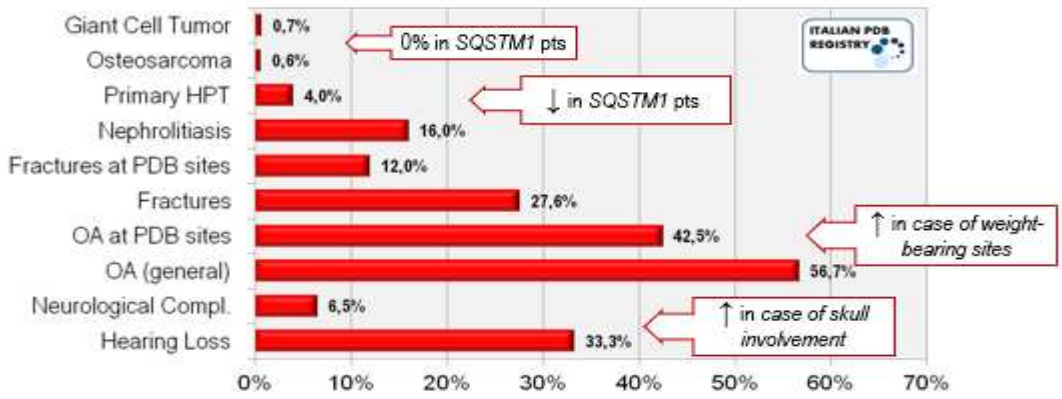

Figure 13: Osteoarthritis occurred in 56.7% of patients and OA at PDB sites in 42.5%. Fractures (27.6%), hearing loss (33.3%), and nephrolithiasis (16%) were frequent. Malignant transformation was rare (osteosarcoma 0.6%, giant cell tumor 0.7%). Genetic Mutations Identified in the Italian Registry of Paget's Disease of Bone

Genetic analysis was performed in a representative subgroup of patients enrolled in the Italian PDB Registry, using Sanger sequencing and next-generation sequencing (NGS) of candidate genes known to be associated with Paget's disease, including SQSTM1, ZNF687, and TNFRSF11A.

DNA was extracted from peripheral blood leukocytes following standardized protocols, and all variants were confirmed by bidirectional sequencing. Among the analyzed patients, SQSTM1 mutations represented the most prevalent genetic alteration, being detected in approximately 13–16% of tested individuals. The majority of these were missense mutations within the UBA and PB1 domains, such as P392L, G425R, and K378X, consistent with the typical mutational spectrum described in European cohorts. Variants in ZNF687 were identified in roughly 5–7% of patients, often in association with a more severe and polyostotic phenotype, earlier age at onset, and increased risk of skull involvement.

Rare mutations in TNFRSF11A (the gene encoding RANK) were found in fewer than 2% of subjects, mostly in familial cases with early-onset disease. Patients harboring SQSTM1 mutations showed higher alkaline phosphatase levels and greater disease extent at diagnosis, but paradoxically, a lower frequency of malignant transformation or hyperparathyroidism, as also indicated in the complication data. Conversely, ZNF687 carriers exhibited more aggressive disease, frequent cranial involvement, and resistance to bisphosphonate therapy in some cases.

Overall, these findings confirm the genetic heterogeneity of Paget's disease in the Italian population and highlight the predominant role of SQSTM1 mutations.

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4.1.5. PDB COMPLICATIONS AND YEARS SINCE DIAGNOSIS

The relationship between disease duration and the occurrence of complications was evaluated. The analysis compared the mean time elapsed since diagnosis in subjects with and without specific complications. As illustrated in **Figure 14**, the presence of complications was consistently associated with a longer disease duration.

Patients with hearing loss, neurological complications, or osteoarthritis at PDB sites had a significantly higher mean number of years since diagnosis compared with those without these manifestations ($P < 0.05$).

A similar pattern was observed for cardiovascular disease (CVD) and calcific valve disease, where disease duration was significantly greater in affected individuals ($p< 0.01$ and $p< 0.05$, respectively).

No significant difference was observed for fractures at PDB sites or nephrolithiasis, suggesting that these complications may occur independently of disease duration.

By contrast, malignant complications, such as osteosarcoma and GCT, tended to develop after a longer disease course, with mean intervals of approximately 10–18 years following initial diagnosis.

Overall, these findings indicate that the risk of major systemic and skeletal complications increases with the duration of PDB, supporting the need for long-term follow-up and monitoring in affected patients. Also in this case, the role of Amino-bisphosphonates could be decisive in blocking the long-term impact of the disease in more of its aspects.

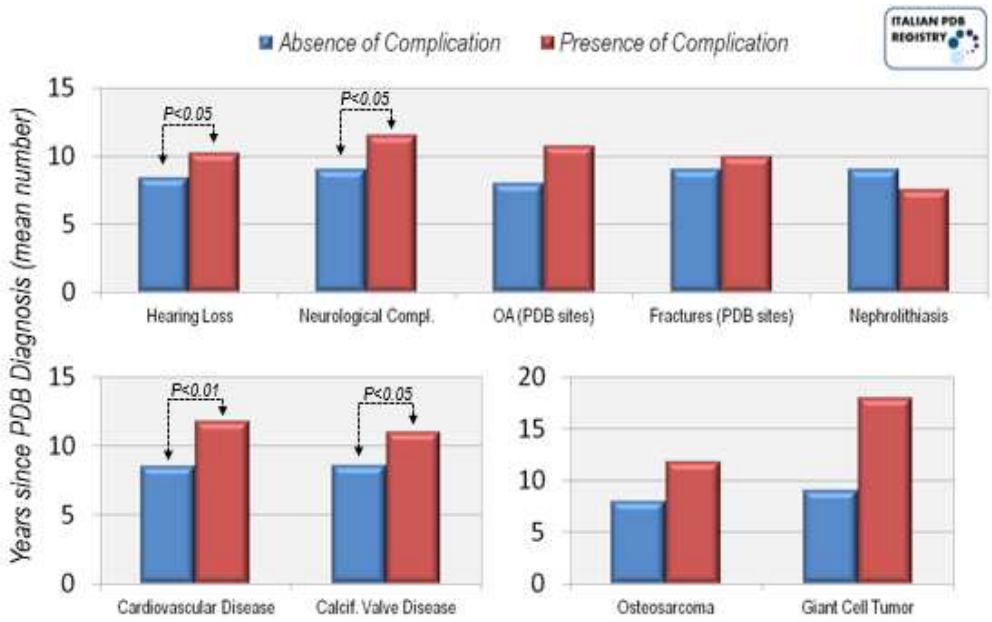

Figure 14. PDB Complications and Years Since Diagnosis Patients with complications such as hearing loss, neurological involvement, or osteoarthritis showed a longer disease duration (P < 0.05).Cardiovascular and valvular disease were also associated with longer PDB duration, whereas fractures and nephrolithiasis were not.

We studied also the relationship between hearing loss and skull involvement. The prevalence of hearing loss was markedly higher in patients with skull involvement (48.9%) compared with those without cranial lesions (30.0%), confirming a strong anatomical association between temporal bone remodeling and auditory impairment. The distribution of hearing loss by decade of birth, showing that the frequency of auditory complications tended to be greater in older birth cohorts (patients born before 1930), whereas a gradual decline was observed in more recent generations.

This trend mirrors the overall decrease in disease severity documented across the registry and suggests that improved diagnosis and earlier therapeutic intervention may have mitigated cranial complications in recent decades.

Together, these findings indicate that skull involvement is a major determinant of hearing loss in PDB, and that this association has persisted across generations, although with decreasing prevalence in younger cohorts. **Figure 15**

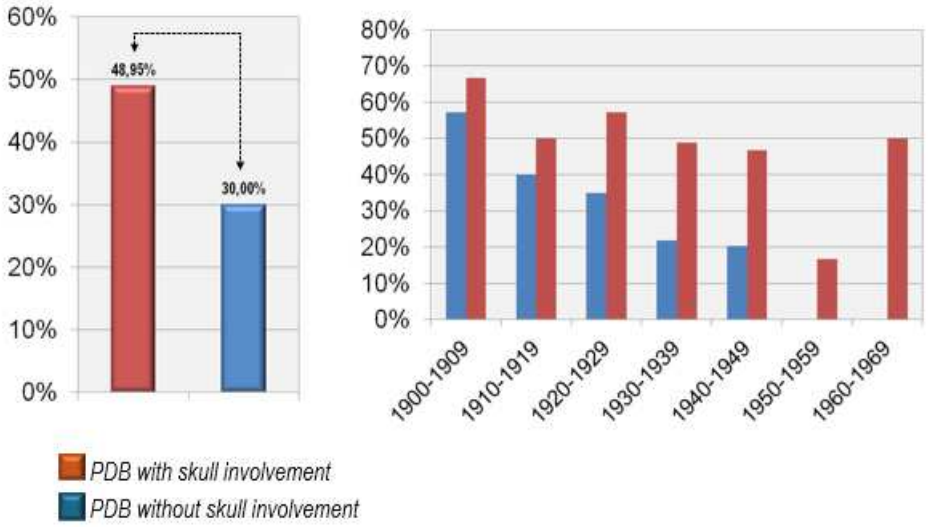

Figure 15. Hearing Loss and Skull Involvement in PDB Hearing loss occurred in 48.9% of patients with skull involvement versus 30% without. Auditory impairment was more frequent in older birth cohorts, reflecting reduced disease severity in recent generations.

4.1.6. CARDIOVASCULAR COMORBIDITIES: COMPARISON WITH SIENA OSTEOPOROSIS POPULATION:

The prevalence of major cardiovascular diseases (CVD) was analyzed in 893 patients with PDB and compared with 1149 controls from the Siena Osteoporosis Study (SiOP).

PDB patients, especially those with polyostotic involvement, showed a significantly higher prevalence of cardiovascular disorders than age- and sex-matched controls. **Figure 16**. Hypertension was the most common finding, affecting 45% of PDB patients and 42% of polyostotic cases, compared with 26% of controls ($P < 0.01$).

A history of myocardial infarction (IMA) was reported in 15% of polyostotic and 12% of all PDB cases, vs 10% of controls ($p< 0.05$). Carotid atherosclerosis was also significantly more frequent in PDB (24%) above all in polyostotic patients (20%) compared with controls (16%; $p< 0.05$). The prevalence of valvular heart disease was more in PDB (16% in polyostotic forms) compared to the controls (21% vs 12 % $p< 0.05$).

A focused analysis identified 7 cases of high-output cardiac failure among 893 PDB patients (0.78%) versus one case in the control population (0.09%), corresponding to a relative risk (RR) of 8.96; 95% CI: 1.10–72.69; $p < 0.05$.

These findings highlight an increased cardiovascular risk profile in PDB, especially in patients with polyostotic disease, likely related to chronic hypervascular bone remodeling and hemodynamic load.

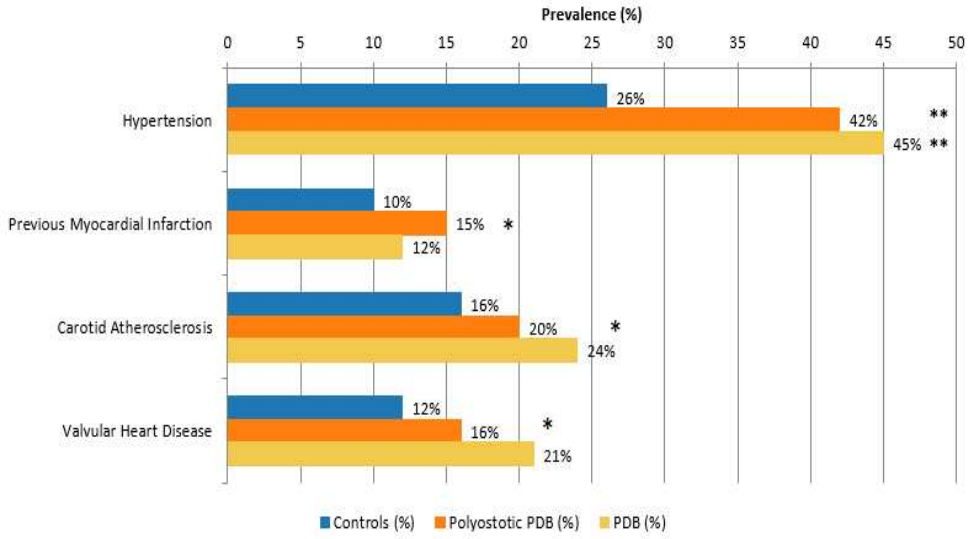

Figure 16 .Cardiovascular Diseases in PDB and Controls. Prevalence of hypertension, myocardial infarction, carotid atherosclerosis, and valvular disease in patients with Paget's disease (PDB) compared with controls from the Siena Osteoporosis Study. Rates were significantly higher in PDB, particularly in polyostotic forms (P < 0.05; P < 0.01*).**

4.2. TASK 2 RESULTS CARDIAC PHENOTYPING AND GENETIC CORRELATIONS IN PAGET'S DISEASE OF BONE

4.2.1. CHARACTERISTICS OF THE POPULATIONS

Seventy-five patients with confirmed Paget's Disease of Bone (mean age 71.9 ± 9.6 years; 72% male, n=54) and 153 control subjects (mean age 70.6 ± 9.1 years; 75% male, n=115) underwent detailed transthoracic echocardiographic assessment according to standardized protocols. Among PDB patients, 59% presented polyostotic disease (n=44), while 41% had monostotic involvement (n=31). Genetic

characterization revealed: 45 patients (60%) were wild-type (WT); 24 harbored SQSTM1/P392L mutations (32%); and 3 carried PFN1 mutations (4%). Among SQSTM1 carriers, the P392L variant was most common (n=19, 79% of SQSTM1+), with smaller numbers of G425R (n=3) and K378X (n=2) mutations. The genetic distribution aligns with previously published European PDB registries. Demographics including age, body mass index (BMI: 26.8 ± 3.2 vs. 27.1 ± 3.5 kg/m²; p = 0.65), and baseline systolic blood pressure (137.4 ± 14.2 vs. 134.8 ± 13.8 mmHg; p = 0.24) were comparable between PDB patients and controls. However, dyslipidemia was significantly more frequent in PDB patients (23%, n=17) compared to controls (7%, n=11; p < 0.001), suggesting either a true disease-associated metabolic phenotype or increased clinical detection. The prevalence of diabetes mellitus was comparable (8% in PDB vs. 6% in controls; p = 0.48). The majority of patients enrolled received bisphosphonate treatment, except for 4 individuals. Specifically, 51 patients (53%) were treated with zoledronate, 18 (24%) with neridronate, and only 2 (2%) with clodronate. **Table 2**

Variable	PDB (n=75)	Controls (n=153)	p value
Age (years)	71.9 ± 9.6	70.6 ± 9.1	0.34
Sex, M/F (n; %)	54/21 (72/28)	114/39 (75/25)	0.70
BMI (kg/m²)	25.1 ± 4.0	25.6 ± 4.2	0.90
BSA (m²)	1.84 ± 0.23	1.82 ± 0.30	0.70
Hypertension (n; %)	30 (40.0)	48 (31.0)	0.10
Atherosclerosis (n; %)	13 (17.3)	12 (7.8)	0.30
Dyslipidemia (n; %)	17 (22.7)	11 (7.2)	0.001
Systolic BP (mmHg)	133.1 ± 13.3	134.8 ± 17.1	0.60
Diastolic BP (mmHg)	73.7 ± 9.6	80.7 ± 10.0	0.01
Heart Rate (bpm)	74.3 ± 7.6	67.0 ± 10.0	<0.001

Table 2. Table 2. Baseline Characteristics of Study Populations, Demographic and clinical characteristics of patients with Paget's Disease of Bone (PDB, n=75) and control subjects (n=153). Data are presented as mean ± standard deviation for continuous variables and number (percentage) for categorical variables.

4.2.2. STRUCTURAL CARDIAC REMODELING: EVIDENCE OF LEFT MASS INCREASE IN PDB

PDB patients demonstrated significant structural cardiac alterations compared to controls, indicative of pathological remodeling. Left ventricular mass index (LVMI), a primary marker of myocardial hypertrophy, was substantially elevated: 111.84 ± 32.52 g/m² in PDB patients versus 91.56 ± 27.22 g/m² in controls ($p < 0.0001$). This 22% increase in LVMI represents pronounced concentric and eccentric remodeling, likely reflecting the chronic hemodynamic burden imposed by increased bone vascularization and elevated cardiac output in PDB. Interventricular septal thickness at end-diastole (IVSd) was significantly increased: 10.97 ± 1.76 mm in PDB versus 9.68 ± 1.34 mm in controls ($p < 0.001$). Similarly, posterior wall diameter at end-diastole (PWd) was elevated: 10.23 ± 1.49 mm in PDB versus 9.55 ± 1.36 mm in controls ($p < 0.0001$). These measurements confirm concentric hypertrophic remodeling as a predominant cardiac adaptation pattern. Left atrial enlargement was also evident, with left atrial volume index (LAVI) significantly higher in PDB patients: 29.28 ± 10.06 mL/m² versus 26.24 ± 7.27 mL/m² in controls ($p < 0.001$). This enlargement suggests chronic exposure to elevated ventricular filling pressures, often associated with diastolic dysfunction, and may predispose to atrial arrhythmias and left atrial thrombus formation. All these findings are shown in **Table 3**

Echocardiographic Parameter	PDB (n=75)	Controls (n=153)	p value
IVSd (mm)	10.97 ± 1.76	9.68 ± 1.34	0.001
PWd (mm)	10.23 ± 1.49	9.55 ± 1.36	<0.0001
LVEDD (mm)	50.41 ± 5.79	49.42 ± 5.81	0.23
LVESD (mm)	30.73 ± 6.41	31.46 ± 5.91	0.39
LVMI (g/m²)	111.84 ± 32.52	91.56 ± 27.22	<0.0001
RWT	0.41 ± 0.1	0.40 ± 0.1	0.48
E/e' average	9.65 ± 2.76	7.56 ± 2.02	<0.0001
E/A	0.95 ± 0.3	0.88 ± 0.2	0.08
Deceleration Time , DT (ms)	233.75 ± 64.28	235.36 ± 59.84	0.87
LAVI (mL/m²)	29.28 ± 10.06	26.24 ± 7.27	0.001
PAPs (mmHg)	26.37 ± 5.62	28.18 ± 8.61	0.1
TAPSE (mm)	21.81 ± 4.32	20.38 ± 3.32	0.006
LVEF (%)	60.25 ± 6.30	59.83 ± 4.88	0.57
Aortic Root (mm)	33.17 ± 4.81	34.62 ± 4.15	0.02
Ascending Aorta (mm)	33.40 ± 4.59	34.03 ± 4.05	0.3
s' wave (cm/s)	15.16 ± 1.87	12.87 ± 2.7	<0.0001
Tricuspid Peak Velocity (m/s)	1.7 ± 0.6	1.6 ± 0.5	0.56
Left Atrium Area (cm²)	19.35 ± 5.37	16.96 ± 4.44	<0.0005
Mitral regurgitation (n; %)	MILD :29 (76%) MODERATE: 8 (24%) SEVERE: 0	MILD: 63 (75%) MODERATE: 21 (25%) SEVERE: 0	0.1
Aortic regurgitation (n; %)	MILD :29 (76%) MODERATE: 8 (24%) SEVERE: 0	MILD: 28 (72%) MODERATE: 10 (26%) SEVERE: 1 (2%)	0.1
Aortic stenosis (n; %)	MILD :4 (90%) MODERATE: 1(10%) SEVERE:0	MILD :0 MODERATE: 1 (100%) SEVERE: 0	0.1

Table 3. Echocardiographic Parameters. Comparative echocardiographic findings in patients with Paget's Disease of Bone (PDB, n=75) and control subjects (n=153). IVSd: interventricular septal diameter; PWd: posterior wall diameter; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; LVMI: left ventricular mass index; RWT: relative wall thickness; LAVI: left atrial volume index; PAPs: pulmonary artery systolic pressure; TAPSE: tricuspid annular plane systolic excursion; LVEF: ejection fraction.

4.2. 3. DIASTOLIC FUNCTION IN PDB: EVIDENCE OF FUNCTIONAL ABNORMALITY

A striking and clinically significant finding was the high prevalence and severity of diastolic dysfunction in PDB patients. The average E/e' ratio—a noninvasive estimate of left ventricular filling pressure—was substantially elevated: 9.65 ± 2.76 in PDB patients compared to 7.56 ± 2.02 in controls ($p < 0.0001$), indicating increased diastolic pressures suggestive of incipient heart failure with preserved ejection fraction (HFpEF).

Diastolic dysfunction of any grade was documented in 45% of PDB patients (36 of 73 evaluable patients) versus 29% of controls (45 of 153; $p < 0.01$). Grade I diastolic dysfunction (impaired relaxation pattern) predominated, present in 40% of PDB patients versus 26% of controls ($p < 0.01$), consistent with early-stage diastolic impairment. Grade II (pseudonormal) filling pattern occurred in 7% of PDB versus 4% of controls ($p = 0.18$). Restrictive (Grade III-IV) patterns were documented in 2% of PDB patients (n=2); no restrictive patterns were observed in controls. **Figure 17**

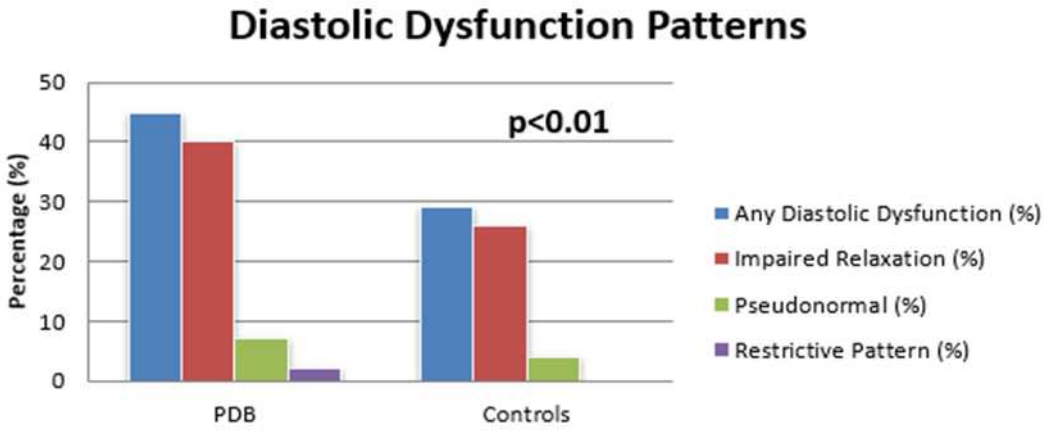

Figure 17 Among the various dysfunctions, concentric remodeling was highly representative and, except for the normal pattern (Any Diastolic Dysfunction), corresponded to the most pronounced diastolic dysfunction.

Age-stratified analysis revealed important trends: among PDB patients ≥70 years (n=42), diastolic dysfunction was present in 55% (n=23) compared to 37% (n=13 of 35) in younger PDB patients, though this difference did not reach statistical significance ($p = 0.20$). However, when hypertensive PDB patients were compared with hypertensive controls of similar age and blood pressure, PDB patients demonstrated significantly worse diastolic parameters: higher E/e' (10.5 ± 2.3 vs. 7.9 ± 1.8; $p < 0.0001$) and LAVI (33 ± 11.3 vs. 29 ± 9.6 mL/m²; $p < 0.0001$). This comparison demonstrates that PDB itself accelerates diastolic dysfunction beyond the effects of hypertension alone, pointing to disease-specific mechanisms.

These findings strongly suggest that diastolic dysfunction is an integral component of the cardiac phenotype in PDB, likely mediated by chronic hemodynamic stress, increased sympathetic activation, and altered myocardial composition (interstitial fibrosis). Early diastolic impairment in asymptomatic patients carries important prognostic implications, as it identifies individuals at heightened risk for progression to symptomatic heart failure. **Figure 18**

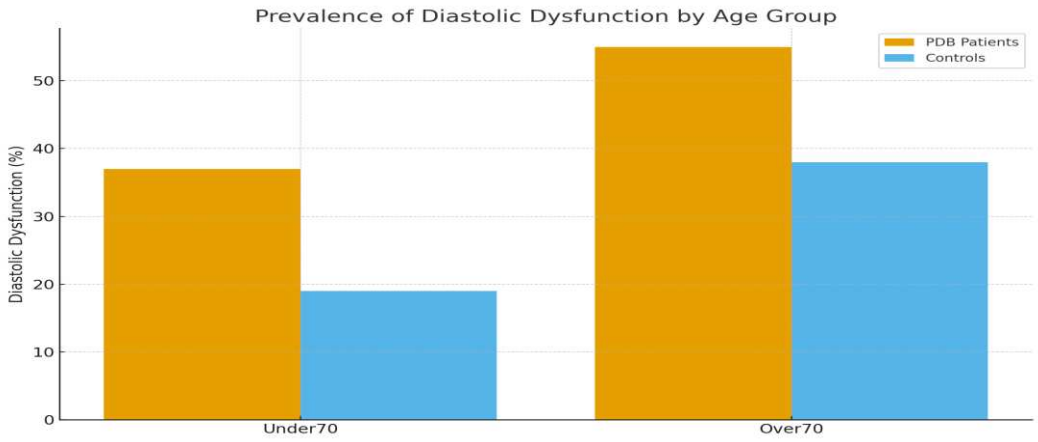

Figure 18. In both groups PDB patients presented a high number of patients with diastolic alterations compared with controls. These findings are not statistically significant differences.

4.2.4. STUDY OF SYSTOLIC FUNCTION

Despite substantial structural remodeling, systolic function remained largely preserved in both PDB and control cohorts. Left ventricular ejection fraction (LVEF) was comparable between groups: 60.25 ± 6.30% in PDB patients versus 59.83 ± 4.88% in controls ($p = 0.57$), with both cohorts maintaining preserved EF (>50% by current diagnostic criteria). This finding contrasts with historical reports suggesting systolic impairment in advanced PDB and likely reflects the benefits of modern bisphosphonate therapy in limiting disease progression.

However, PDB patients demonstrated enhanced right ventricular systolic performance, consistent with a hyperdynamic cardiac state. Tricuspid annular plane systolic excursion (TAPSE), a marker of right ventricular systolic excursion, was higher in PDB patients (21.81 ± 4.32 mm) compared to controls (20.38 ± 3.32 mm; $p = 0.006$). Similarly, tricuspid systolic annular velocity (s' wave on tissue Doppler imaging) was significantly elevated in PDB: 15.16 ± 1.87 cm/s versus 12.87 ± 2.70 cm/s in controls ($p < 0.0001$). This pattern of enhanced RV performance reflects a compensatory hyperdynamic state, likely driven by chronic peripheral hyperflow from increased bone vascularization and the "steal" of blood flow to pagetic bone.

4.2.5. CARDIAC REMODELING PATTERNS: OVERALL PREVALENCE AND DISTRIBUTION

Overall, pathological cardiac remodeling was significantly more prevalent in PDB patients (67%, n=50 of 75) compared to controls (47%, n=72 of 153; $p < 0.05$). Remodeling was classified according to standard criteria based on LVMI and relative wall thickness (RWT): Concentric remodeling (increased RWT without increased LVMI) occurred in 19% of PDB patients (n=14) versus 23% of controls (n=35), representing normal remodeling without hypertrophy.Concentric hypertrophy (increased both RWT and LVMI) was significantly more common in PDB patients: 27% (n=20) versus only 9% in controls (n=14; $p < 0.0001$).

This pattern indicates the most pathologically significant remodeling geometry, associated with diastolic dysfunction and increased cardiovascular risk.Eccentric hypertrophy (increased LVMI with normal or decreased RWT) occurred in 17% of PDB patients (n=13) versus 16% of controls (n=24), representing similar prevalence in both groups.Normal geometry (normal LVMI and RWT) was preserved in 33% of PDB patients (n=25) versus 53% of controls (n=81; $p < 0.001$).The predominance of concentric hypertrophy in PDB patients represents a pathologically significant remodeling pattern, distinguished from the predominantly normal geometry observed in age-matched controls. **Figure 19**

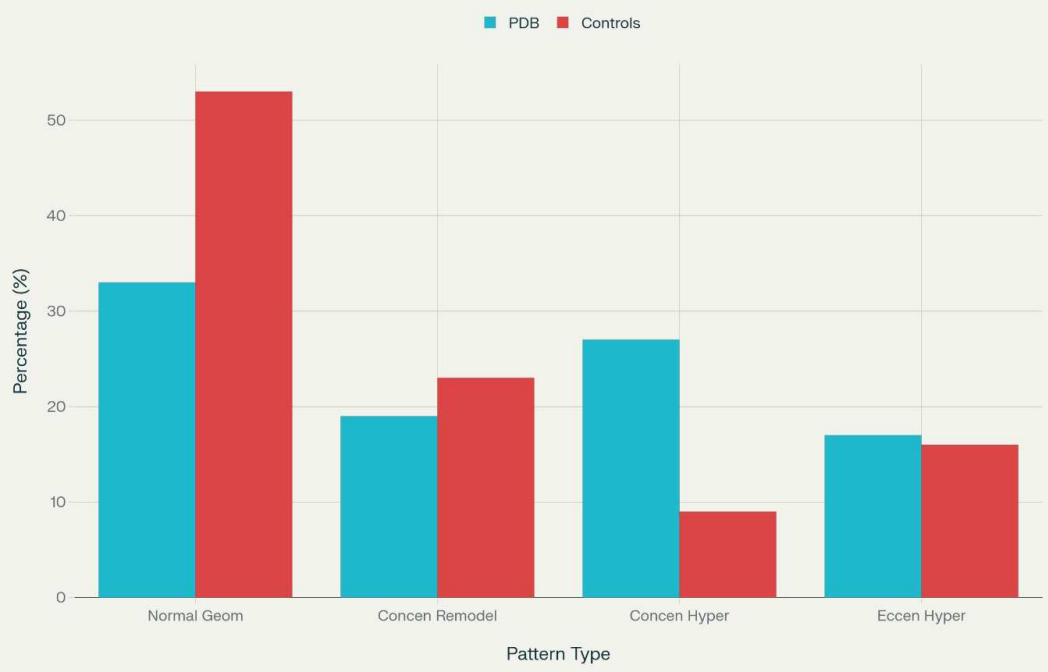

Figure 19. Distribution of cardiac remodeling patterns in patients with Paget's Disease of Bone (PDB) compared to age-matched controls. The chart illustrates the percentage of subjects exhibiting normal geometry, concentric remodeling, concentric hypertrophy, and eccentric hypertrophy. Notably, concentric hypertrophy is significantly more prevalent in PDB patients ($p < 0.0001$) , indicating pathologically significant myocardial remodeling associated with increased cardiovascular risk, while normal geometry predominates among controls. Overall cardiac remodeling was more common in PDB ($p < 0.05$)

4.2.6. HYPERTENSIVE PATIENTS COMPARATION

We compared PDB patients with hypertension compared to age-matched hypertensive controls. Our analysis revealed that PDB patients had significantly higher values of E/e' average (10.5 ± 2.3 vs. 7.9 ± 1.8, $p < 0.0001$) and LAVI (33 ± 11.3 vs. 29 ± 9.6

mL/m², p < 0.0001) compared with controls. Diastolic function analysis demonstrated a higher prevalence of diastolic dysfunction among PDB patients. Specifically, Grade I dysfunction was observed in 17/28 PDB patients (61%) vs 15/48 controls (31%), Grade II in 2/28 (7%) versus 2/48 (4%), and Grade III in 2/28 (7%) versus 0/48 (p < 0.005). Only 7/28 PDB-hypertensive patients (25%) exhibited a normal diastolic pattern, compared with 31/48 (65%) controls with hypertension (p<0.0001). These preliminary data suggest that, under similar conditions of hypertension, PDB patients may experience an earlier progression of diastolic dysfunction compared to hypertensive controls. **Figure 20**

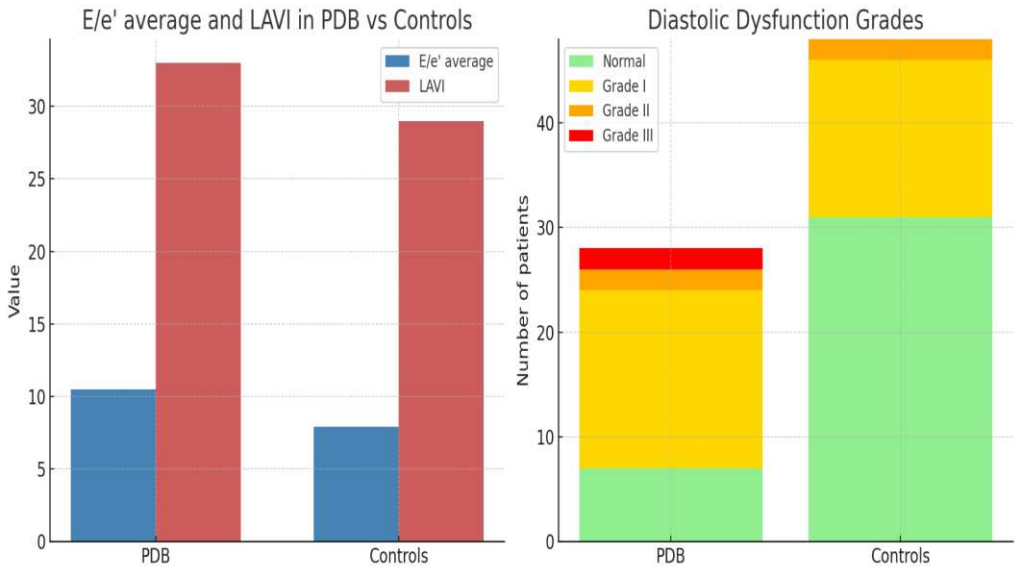

Figure 20. Difference in echocardiographic parameters between hypertensive patients of both the populations. PDB presented increased values of LAVI and E/e' avg (p<0.0001) . PDB-hypertensive presented a high number of patients by diastolic dysfunction compared with age matched hypertensive controls

4.2.7. GENOTYPE-PHENOTYPE CORRELATIONS

Analysis of cardiac phenotypes stratified by genetic status revealed distinctly different cardiac adaptation patterns among mutation carriers versus wild-type patients, providing novel insights into genotype-phenotype correlations in PDB.

Wild-type (WT) Patients (n=45, 60%)

Wild-type PDB patients demonstrated the most pronounced cardiac remodeling and dysfunction. Concentric hypertrophy was present in 20 of 45 wild-type patients (44%), the highest prevalence among all genetic groups ($p < 0.01$). LVMI was elevated at 118.4 ± 34.2 g/m², and diastolic dysfunction was documented in 51% (23 of 45). Mean E/e' ratio was 10.2 ± 2.8, indicating moderately elevated filling pressures.

SQSTM1 Mutation Carriers (n=24, 32%)

SQSTM1 carriers exhibited a distinctly attenuated remodeling phenotype, suggesting a protective or modulatory effect of the mutation. Overall remodeling prevalence was 50% (12 of 24) versus 71% in wild-type patients ($p = 0.003$). Strikingly, concentric hypertrophy was absent in all SQSTM1 carriers (0 of 24), creating a statistically significant difference compared to 44% prevalence in wild-type patients ($p < 0.0001$).Instead, SQSTM1 carriers more frequently exhibited concentric remodeling (29%, n=7) and eccentric hypertrophy (21%, n=5), patterns generally associated with better functional outcomes than concentric hypertrophy. Diastolic dysfunction was present in 42% (10 of 24), slightly lower than wild-type patients, and the mean E/e' ratio was 9.1 ± 2.4, lower than wild-type ($p = 0.08$, not statistically significant but clinically meaningful).Notably, despite the presence of SQSTM1 mutations and associated skeletal disease severity, both systolic function (LVEF = 61.2 ± 5.8%; $p = 0.45$ vs. controls) and diastolic parameters remained relatively preserved compared to wild-type PDB patients. This pattern suggests that the SQSTM1 mutation may impose a protective effect on cardiac hemodynamics, possibly through altered inflammatory signaling or autophagy-related cellular adaptation mechanisms. **Figures 21 and 22**

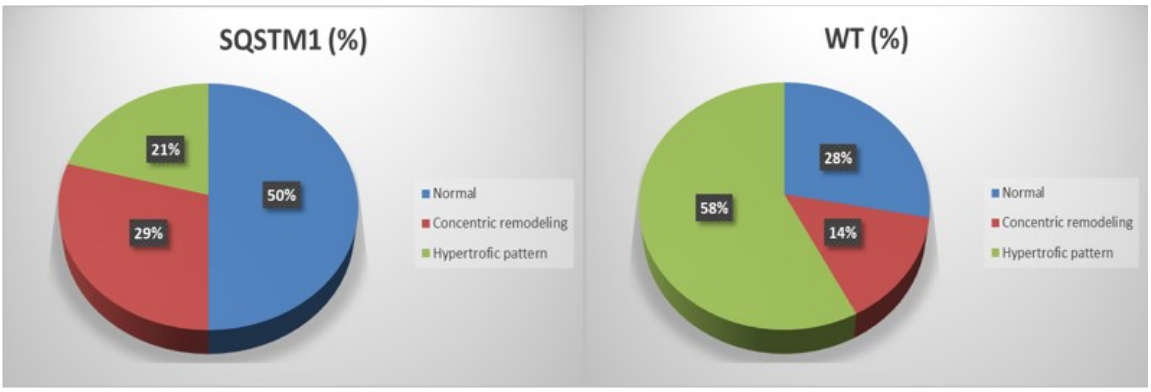

Figure 21. Comparison of cardiac remodeling patterns between SQSTM1-mutated and wild-type groups (WT). Most patients carrying the SQSTM1 mutation exhibited a normal cardiac remodeling pattern, whereas wild-type patients more frequently showed an increased hypertrophic profile.

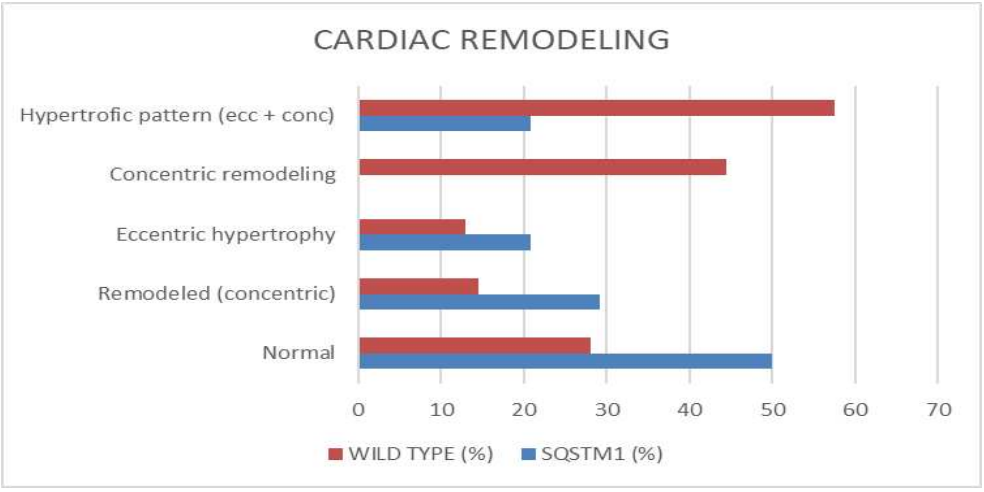

Figure 22. Cardiac remodeling patterns in SQSTM1-mutated versus wild-type patients. The bar chart shows the distribution (%) of hypertrophic patterns (eccentric + concentric), concentric remodeling, eccentric hypertrophy, concentric remodeling, and normal geometry for each genetic group. SQSTM1 patients demonstrate a higher prevalence of normal geometry and concentric remodeled patterns, while wild-type patients have higher rates of concentric and hypertrophic remodeling.

PFN1 Mutation Carriers (n=3, 4%)

The three PFN1 carriers (all female; mean age 61.67 ± 10.08 years) exhibited a striking and distinct hyperdynamic cardiac phenotype, markedly different from both wild-type and SQSTM1 patients. All characteristics of the patients are resumed on **Table 4.**

Characteristic	PFN1 Patients (n=3)
Sex (M/F)	F = 3 (100%)
Age (years)	61.67 ± 10.08
ALP (U/L)	367 ± 383.2
Ca^{2+} (mg/dL)	10.1 ± 3.2
Phosphate (mg/dL)	3.6 ± 1.5
25-OH Vitamin D (ng/mL)	42 ± 12
PTH (pg/mL)	51.2 ± 18
Creatinine (mg/dL)	0.7 ± 0.1
IVSd (mm)	9.7 ± 0.1
PWd (mm)	10 ± 1.6
LVEDD (mm)	44.3 ± 4.1
LVMI (g/m^2)	99 ± 24.1
RWT	0.46 ± 0.3
E/e' average	6.3 ± 1.24
E/A	1.21 ± 0.3
LAVI (mL/m^2)	27.3 ± 4.1
PAPs (mmHg)	20.7 ± 1
TAPSE (mm)	25 ± 3.8
s' wave (cm/s)	15.3 ± 1.25
LVEF (%)	72.7 ± 4.5
Aortic Root (mm)	26.3 ± 2.5
Ascending Aorta (mm)	28.3 ± 2.6

Table 4. Characteristics and Echocardiographic Findings of PFN1-Mutated PatientsClinical and echocardiographic features of the three patients carrying PFN1 mutations. All patients were female with monostotic disease. Data are presented as mean ± standard deviation

Left ventricular ejection fraction was markedly elevated at 72.7 ± 4.5%, significantly higher than controls (59.82 ± 6.3%; $p < 0.01$) and wild-type PDB patients. Global longitudinal strain (GLS) was substantially enhanced (mean –23.4 ± 2.1% vs. normal reference –18% to –22%), indicating supranormal systolic performance. **Figures 23 and 24**. Stroke volume was at the upper normal limit (75 ± 7.8 mL), and cardiac output was preserved (4.5 ± 2.2 L/min). Critically, diastolic function remained normal in all three PFN1 carriers: mean E/e' ratio = 6.3 ± 1.24 (well below the elevated threshold), and no patient demonstrated diastolic dysfunction despite high bone turnover.This hyperdynamic phenotype represents a fundamentally different cardiac adaptation to PDB compared to wild-type and SQSTM1 patients. The enhanced systolic performance without diastolic impairment suggests a potentially compensatory adaptation to the skeletal hyperactivity

characteristic of PFN1-related PDB, wherein profilin-1 mutations drive enhanced osteoblastic activity and bone remodeling, necessitating a more robust cardiac compensatory response. While the sample size is limited (n=3), this finding is novel and clinically significant, suggesting that PFN1 mutations confer a distinct and potentially more favorable cardiac phenotype with preserved diastolic function.

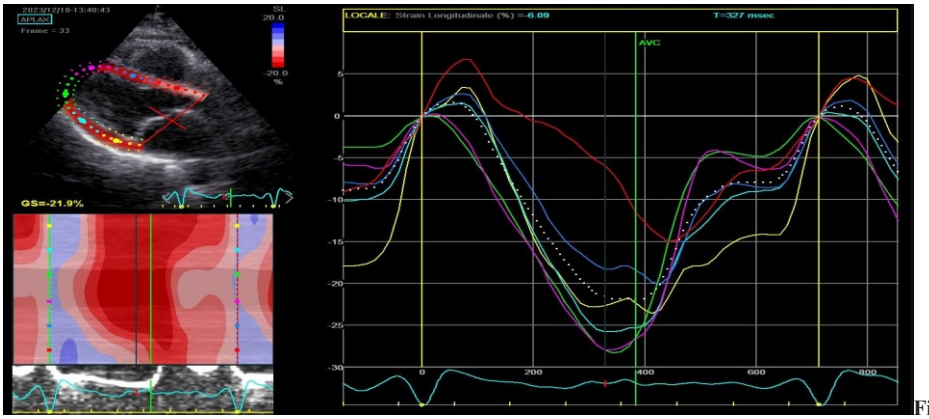

Figure 22 : Increased values of global longitudinal strain in PFN1 patient, at level of parasternal-long axis projection (PLAX), in particular in apical-septal segment

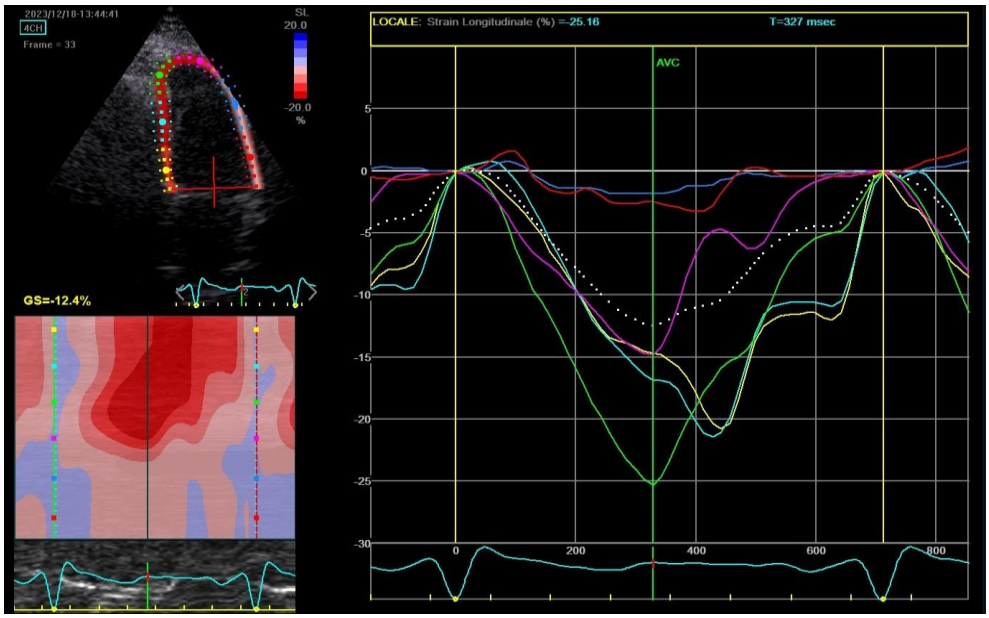

Figure 23. Global longitudinal strain analysis in the two-chamber (2CH) view of a PFN1-mutated patient showing increased speckle-tracking activity across all basal and apical segments, with a marked enhancement in the apical septal region.

4.2.8. VALVULAR CALCIFICATIONS AND DYSFUNCTION AND HEMODYNAMIC ASSESSMENT

Valvular sclerosis and calcification emerged as clinically significant findings, occurring at substantially elevated prevalence in PDB patients. Aortic valve calcification was detected in 29% of PDB patients (n=22) versus only 5% of controls (n=8; $p < 0.000001$), representing a 5.8-fold increase in prevalence. Mitral valve calcification was similarly elevated: 21% in PDB patients (n=16) versus 6% in controls (n=9; $p < 0.0002$), representing a 3.5-fold increase. Notably, genetic status influenced the prevalence of valvular calcification. Among PFN1 mutation carriers (n=3), aortic valve calcification was present in 67% (n=2), followed by wild-type patients at 32% (n=14 of 45), with SQSTM1 carriers at 25% (n=6 of 24). This pattern suggests that PFN1-related bone hyperactivity may promote systemic calcification processes, though the small sample size limits definitive conclusions.Importantly, no cases developed hemodynamically significant aortic stenosis or mitral regurgitation requiring clinical intervention. Maximum aortic jet velocity in calcified valves ranged from 1.6 to 2.2 m/s (all <2.5 m/s, indicating no significant stenosis), and mitral regurgitation was mild in all cases. This observation suggests that modern bisphosphonate therapy, by limiting bone turnover and circulating calcium-phosphate disturbances, may effectively prevent progression to functionally significant valvular disease.

Pulmonary artery systolic pressure (PAPs), estimated from tricuspid regurgitation velocity when present or assumed normal when absent, was comparable between groups: 26.37 ± 5.62 mmHg in PDB patients versus 28.18 ± 8.61 mmHg in controls ($p = 0.1$). This finding indicates that systemic pulmonary hypertension is not a prominent hemodynamic feature of early-to-moderate PDB, contrasting with historical descriptions of advanced disease. The preservation of normal pulmonary pressures in modern-treated cohorts likely reflects the effects of bisphosphonate-based therapy in limiting disease progression.

CHAPTER 5: DISCUSSION

Paget's Disease of Bone is a focal metabolic disorder of the skeleton that primarily affects elderly individuals. This condition can lead to the onset of complications at the osteoarticular, metabolic, and cardiovascular levels. Our study provides a detailed evaluation of cardiac structure and function in PDB patients, highlighting patterns of remodeling and diastolic dysfunction that may precede overt heart disease. A key finding of our work is the presence of left ventricular remodeling in PDB patients. Echocardiography revealed increased interventricular septal thickness (IVS), relative wall thickness (RWT), and left ventricular mass index (LVMI) compared to matched controls. These changes indicate an increased trend for both concentric and eccentric hypertrophic adaptation, likely reflecting chronic exposure of the heart to systemic alterations associated with PDB. While historical reports suggested high-output states in advanced disease, our cohort (carefully selected to exclude pre-existing structural cardiac abnormalities) demonstrates that remodeling occurs even in early or moderate disease, despite an adequate treatment, suggesting that PDB may subtly alter myocardial structure over time through chronic hemodynamic and possibly paracrine skeletal influences. Diastolic function was markedly impaired in our PDB cohort. Elevated average E/e′ , increased left atrial volume index (LAVI), and abnormal mitral inflow patterns were observed, indicating increased left ventricular filling pressures and reduced relaxation capacity. This diastolic impairment was consistently worse in PDB patients than in controls, highlighting that diastolic dysfunction is an early and predominant feature of cardiac involvement in PDB. The combination of concentric remodeling and increased filling pressures could suggest a pathophysiological continuum in which skeletal hyperactivity, vascular changes, and systemic effects collectively contribute to myocardial stiffness. In our study, no significant difference in LVEF was observed between the two populations. Interestingly, unlike previous reports, controls exhibited slightly lower LVEF values compared to PDB patients. As previously noted, minimal variations in LVEF on echocardiography do not necessarily indicate impaired systolic function. Indeed, considering that LVEF measurement is semi-quantitative and relatively imprecise, and that values in both groups were above

50%, corresponding to preserved ejection fraction, no evidence of systolic dysfunction can be inferred. More sensitive techniques, such as global longitudinal strain, would be required to detect subtle impairments [56]; however, these were not the primary focus of our study. This study also confirms a higher prevalence of valvular sclerosis and calcification in PDB patients, particularly affecting the aortic and mitral valves. However, patients were asymptomatic and did not exhibit severe valvular abnormalities. We may hypothesize that early use of bisphosphonates could contribute to halting the progression of valvular calcification, thereby preventing worsening of valvular disease, although we have no direct evidence to support this assumption.The potential influence of genetic mutations such as SQSTM1/P392L and PFN1 on cardiac function is intriguing. Our findings indicate that carriers of SQSTM1 mutations exhibit a lower overall prevalence of left ventricular remodeling compared to wild-type patients. Interestingly, the type of remodeling appears to differ concentric remodeling is more frequent in SQSTM1 carriers, whereas concentric hypertrophy is notably absent, and eccentric hypertrophy is observed in a subset of patients. Despite these structural variations, no significant impairment of systolic or diastolic function was detected in SQSTM1 mutation carriers, suggesting that these cardiac alterations are largely subclinical. These results are in line with recent findings in vivo about P392L mutation and these can open new scenarios in phenotype of these patients. More studies in humans were needed to investigate these characteristics. Overall, our results support the notion that SQSTM1 mutations may shape the pattern of cardiac remodeling in PDB rather than increase the overall risk of structural or functional cardiac damage and this aspect can be positive in exercise tolerance and health status in the patients. Regarding PFN1 mutations, patients exhibit a distinctive cardiac phenotype characterized by hyperdynamic systolic function. Despite the monostotic involvement and the absence of major comorbidities, PFN1 carriers demonstrated markedly elevated left ventricular ejection fraction and increased global longitudinal strain, alongside preserved stroke volume and cardiac output. Importantly, diastolic function remained normal, suggesting that the hyperkinetic pattern is not associated with impaired filling or elevated ventricular pressures. These findings may reflect a compensatory cardiac adaptation to chronic peripheral hyper flow resulting from

increased bone turnover in PDB. The combination of enhanced systolic performance indicates that the myocardium in PFN1 patients can sustain elevated hemodynamic demands, but the impact of this adaptation over the years is mostly unknown. While the sample size is small, this hyperdynamic pattern contrasts with the more subtle, often subclinical cardiac remodeling observed in wild-type and SQSTM1 mutation carriers, highlighting a potential genotype-specific cardiac response in PDB. Further longitudinal studies in larger cohorts are warranted to confirm whether this hyperkinetic phenotype persists over time and to investigate its clinical implications, including potential progression to pathological remodeling or cardiovascular complications. Despite its strengths, our study has several limitations. The study was conducted at a single institution, which may introduce referral and selection bias, and could impact the generalizability of results to other populations. Echocardiography, while widely available and clinically practical, is operator-dependent and may be less accurate than cardiac magnetic resonance (CMR) for quantifying myocardial mass, volumes, and tissue characterization. Furthermore, exercise capacity and cardiac performance under stress were not evaluated; incorporating treadmill or stress testing could provide a more complete understanding of functional impairment. Our study was cross-sectional; while it identifies structural and functional differences, it cannot establish the temporal progression of cardiac involvement. A longitudinal follow-up, which we plan over five years, will be crucial to assess progression and the impact of therapy. Finally, biomarkers such as pro-BNP could help detect early diastolic stress and identify patients at risk of developing heart failure with preserved ejection fraction (HFpEF), but were not included in the present study. **SynTable**

Aspect	Summary and Judgment
Rationale	Clarify cardiac consequences of Paget's disease of bone and assess genetic influences (SQSTM1, PFN1) on remodeling and outcomes.
Background	Previous research suggested possible cardiac effects in PDB, but mechanisms and genetic roles were unclear.
Methods	Cohort study using standardized echocardiographic evaluation and genetic testing; robust protocol with strict criteria.
Results	Cardiac remodeling is prevalent in PDB, with SQSTM1 mutations linked to more distinct LV changes; PFN1 mutations less frequent.
Innovations	First study combining echo and genetic profiling in PDB; mutation-specific cardiac remodeling patterns identified.
Limitations	Single-center, cross-sectional design; small genetic subgroups; echocardiography only; strict patient exclusions; operator bias.
Future Perspectives	Multicenter, longitudinal studies needed; integration of genetic/imaging protocols; towards personalized risk and management.

SynTable. This synoptic table summarizes the core elements of the thesis, providing a comprehensive overview of rationale, methodology, findings, innovations, limitations, and future research directions.

CHAPTER 6: CONCLUSIONS

In conclusion, this study advances understanding of cardiac involvement in Paget's disease of bone (PDB) by revealing that both clinical and genetic factors—particularly SQSTM1 mutations—significantly influence cardiac remodeling patterns. The findings underscore the distinctive myocardial adaptation associated with specific genetic backgrounds, highlighting how mutations in genes such as SQSTM1 and PFN1 may drive variability in cardiac structure and function among affected patients. The use of detailed echocardiographic assessment has demonstrated its potential for early identification of subclinical cardiac changes in PDB, supporting the role of imaging as a valuable screening tool. Importantly, this work opens new avenues for translational research aimed at decoding the molecular interplay between bone metabolism, genetics, and cardiovascular health. Understanding these relationships could pave the way for more personalized monitoring and management strategies in PDB, including targeted screening programs and risk stratification in genetically at-risk individuals. Despite the novel insights generated, the study also demonstrates the necessity of further multicenter, prospective, and longitudinal investigations to validate these observations and to more precisely elucidate their prognostic significance and therapeutic implications. Ultimately, integrating genetic testing with advanced imaging protocols may be key to improving clinical outcomes for patients with Paget's disease of bone.

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