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REVIEW PAPER

The Clinical Relevance of the Bone Vascular System: Age-Related Implications

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Abstract

]The microcirculation of bone and marrow is vital for bone development, maintenance, and repair. In addition to the well-known function of transporting oxygen, nutrients, systemic hormones, precursor cells, waste, etc., the bone vascular network plays a role in the mechanical induction of bone formation. In addition, arteries and marrow sinusoids are critical components of hematopoietic stem cell niches. This review discusses the various roles of the bone and marrow microcirculation in regard to (1) bone development, remodeling, and fracture repair; (2) the regulation of bone intramedullary pressure and interstitial fluid flow; and (3) the mobilization of mature blood cells into the peripheral circulation. Age-associated dysfunction of the microcirculation is discussed in relation to how it may disturb bone and marrow homeostasis, fracture repair, and organismal vitality. Finally, the review invites the reader to consider the efficacy of treatments designed to alleviate bone and marrow pathologies in the face of a compromised vascular network.

Keywords Microcirculation · Bone · Marrow · Blood flow · Blood cells

Introduction

Highways and transit systems permit the passage of people and commerce, with lane closures, road closures, and road blocks slowing this transport. The vascular network is equivalent to the highways and transit systems in that it carries white blood cells, red blood cells, platelets, systemic hormones, nutrients, oxygen, carbon dioxide, waste products, precursor cells, etc. (i.e., goods and services) along its route. Cells and factors are carried to and from the peripheral circulation, originating in certain tissues and traveling to target tissues. As we age and/or develop disease, vascular function declines [1–3]. Age- and disease-related pathologies of bone blood vessels, in particular, include diminished endothelium-dependent vasodilation [4–8], vascular rarefaction [9, 10], augmented vascular calcification [9], and arteriosclerosis/atherosclerosis [11–13].

In regard to the transit of good and services, declines in vasodilator function, vascular rarefaction and calcification, and arteriosclerosis/atherosclerosis of bone blood vessels are corollary to lane closures, road closures, and road blocks. All of these vascular ailments serve to reduce and/or impede the passage of blood and, in essence, diminish or obstruct the delivery of blood cells (i.e., white and red blood cells, platelets, and precursor cells), systemic hormones, nutrients, and oxygen, and attenuate the removal of tissue waste. In fact, diminished bone and marrow blood flow and perfusion are a common occurrence in old age and has been demonstrated in animal models and humans [4, 6, 14–16]. From this perspective, we can appreciate how bone vascular dysfunction contributes to bone and marrow pathology. Under this premise, we must further appreciate how bone vascular dysfunction may encumber efforts to treat bone and marrow ailments. In other words, medications and treatments designed to correct or assuage bone and marrow pathologies often rely upon the vascular system for its transport into the skeletal system. This begs the question as to how effective is the transport of goods and services (i.e., medications and treatments) if the highways and transit systems are in disrepair. This review will highlight three main facets as to why we should investigate the bone vascular system, how blood vessels change with advancing age, and how this impacts fracture repair and marrow function.

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Bone Development and Repair Require a Vascular Supply

The interaction between osteoblasts, vascular endothelial cells, and other cells within the bone microenvironment modulates development and remodeling [17]. The theory that vascular endothelial cells and/or pericytes convert into pre-osteoblasts or osteoblasts suggests the direct involvement of blood vessels in bone formation [17]. During bone development, remodeling, and regeneration, the formation of new blood vessels (i.e., angiogenesis) is a key regulating factor [18, 19]. In fact, vascular ingrowth is necessary for both endochondral and intramembranous ossification [20]. The presence or absence of a pre-existing cartilage template and the site of bone development distinguishes the two types of ossification. During embryogenesis, intramembranous and endochondral ossification typically occur in the axial and appendicular skeleton, respectively, and form the flat and tubular bones, respectively [21]. Both, however, rely upon a vascular supply.

Intramembranous ossification occurs without a pre-existing cartilage template and commences with an avascular mesenchymal condensation [21]. While the role of blood vessels during this type of ossification is not frequently studied [18], the differentiation of pre-osteoblasts into secretory osteoblasts occurs concomitant with blood vessel invasion of the bone anlage [22]. In models of distraction osteogenesis, which transpires primarily via intramembranous mechanisms [17], bone formation occurs only at sites close to blood vessels [23]. In the distraction gap, bone formation occurred in close proximity to the recently developed and large (150–200 μm in diameter) vascular sinuses [17]. It has been postulated that vascular cells or cells close to blood vessels have osteogenic properties or secret osteogenic factors [24–27]. In elderly patients following vertical distraction of the mandible, instances of scarce vascularization were coupled with poor bone formation [18]. As anticipated, blood supply to the site of bone formation is augmented during distraction osteogenesis, as evidence of this measure via various techniques (e.g., vascular corrosion casts [28] and scintigraphic investigations [29, 30]). In addition, regional blood flow to bone-forming sites has been demonstrated to increase 10-fold above control conditions [17], remaining 3-fold higher vs. control up to 17 weeks post-corticotomy [31–33].

During endochondral ossification, bone replaces an existing calcified cartilage template [34], only after invasion by new capillaries [35]. The cartilage template is established by chondrocytes that eventually hypertrophy and secrete an extracellular matrix containing collagen X [36]. Hypertrophic chondrocytes release vascular endothelial cell growth factor (VEGF) to initiate blood vessel development and the newly established blood supply brings in nutrients [37] and presumably brings in osteogenic cells [18, 37]. Pre-osteoclasts and chondroclasts remove the extracellular matrix secreted by the hypertrophic chondrocytes [36, 37] and blood vessel invasion delivers osteoblasts to lay down the extracellular matrix of bone [36]. In concert with VEGF, osteoclasts and matrix metalloproteinase-9 work to ensure proper bone development [36]. Inactivation of VEGF during endochondral ossification in a murine model altered the vascular morphology within the growth plate, eliminating proper growth and invasion of the metaphyseal vascular supply [37]. Thus, vascular invasion of the cartilage template, as mediated by VEGF, serves as the linchpin between resorption of the template and formation of bone [37]. Further, it has been recently speculated that bone blood vessels serve as a guide for (1) the collagen template, (2) the replacement of the template with bone, and (3) bone morphogenesis [38]. For example, calcein labeling (i.e., bone formation) was observed in close proximity to blood vessels during endochondral ossification in developing mice, leading the authors to conclude that the blood vessels served as a template for mineral deposition [38]. In the same series of investigations, VEGF overexpression in osteoblasts altered the vascular pattern, increased the number of blood vessels, and caused vascular over-sprouting at the bone circumference [38]. Interestingly, enhanced bone formation was observed at the bone circumference, next to the blood vessels [38].

Recent investigations have shed more light on the interrelationship between bone blood vessels and bone cells. For example, co-invasion of pre-osteoblasts and blood vessels into the cartilage template during bone development and into the cartilaginous callus during fracture repair has been observed [39]. Further, angiogenesis related to the development of long bones in mice was associated with Notch signaling [40]. When Notch signaling was experimentally disrupted, vascular defects in morphology and growth occurred, as well as bone abnormalities (i.e., diminished osteogenesis and bone length, chondrocyte deficiencies, a decreased number of trabeculae, and reduced bone mass) [40]. Restoration of Noggin, an angiocrine factor secreted from endothelial cells and regulated by Notch, reversed the vascular defects and bone abnormalities [40]. In addition, marrow capillaries and sinusoids in genetically modified and aged mice were distinguished based upon their expression of CD31 and endomucin (Emcn) [41]. Based upon this categorization, endothelial cells associated with capillaries stained high for CD31 and Emcn, while those in sinusoids stained low for CD31 and Emcn [41]. Thus, endothelial cells in bone were termed type H for the $CD31^{hi}/Emcn^{hi}$ subpopulation (i.e., capillaries) and type L for the $CD31^{lo}/Emcn^{lo}$ subpopulation (i.e., sinusoids) [41]. Interestingly, osteoprogenitor cells (i.e., $Osterix^{+}$, $Runx2^{+}$, and collagen $1\alpha^{+}$), which can eventually differentiate into osteoblasts, were preferentially located next to the type H as opposed to the type L blood vessels, despite the low prevalence of type H endothelial cells in relation to the total population within the marrow [41]. These data suggest a direct

relationship between endothelial cells and pre-osteoblasts during bone growth. Further, 7 days following irradiation in C57BL/6J mice, tibiae had a high and low number of type H and type L endothelial cells, respectively, suggestive of a role for H type blood vessels in neo-angiogenesis [41].

Following intramembranous and endochondral ossification during development, the skeletal system becomes highly vascularized [42]. Beyond skeletal maturity, bone resorption and formation continues under a regulated fashion to maintain strength and integrity. This is achieved by bone remodeling, which is under the control of basic multicellular units of cortical bone [43] or bone remodeling compartments of trabecular bone [44]. Basic multicellular units and bone remodeling compartments consist of osteoclasts, osteoblasts, and an always present blood vessel [45–47]. The blood vessel provides nutrients, oxygen, and precursor cells to the bone-remodeling site, illustrating the importance of the vascular supply for bone homeostasis.

Partial or complete breaks cause a discontinuity in bone and represent fracture. Often there is a disruption in the vascular supply surrounding the fracture site. Bone healing commences in an attempt to restore homeostasis and this occurs via mechanisms of endochondral ossification [17]. Under these circumstances, a callus forms over the fracture site where angiogenesis will occur [18] and blood vessel formation at the callus allows for the replacement of cartilage with bone [48]. When blood delivery reaches its nadir, the transports of products necessary for bone repair are enhanced [49]. For example, blood flow was enhanced 0–14 days following osteotomy and returned to baseline by 21–28 days, coinciding with mineral deposition at the fracture site [49, 50]. A review of the literature in regard to the vascularization of human long bones revealed disparate zones of blood vessel density in femoral and tibial shafts [51]. When divided into three zones (i.e., the upper third, middle, and lower third), femora had moderate, high, and poor vascularization, respectively, and tibiae had high, moderate, and poor vascularization, respectively [51]. The authors speculated that these disparate zones of vascularization could contribute to fracture non-union [51], which occurs at high rates in femora and tibiae [52]. Once again, the vascular supply is key. Under all four paradigms (i.e., intramembranous ossification, endochondral ossification, bone remodeling, and fracture healing), the vascular supply is requisite.

The Fluidic Nature of Bone Ensures Its Dependence upon the Vascular System

The skeletal system is highly vascularized and porous, lending itself to a high fluid content. The vascular system of bone contains afferent vessels, capillaries and sinusoids, and efferent vessels [53, 54]. Ionic exchange [53] and filtration [55] occur in the capillaries and sinusoids, with capillaries located in yellow (fatty) marrow and the sinusoids located in red (hematopoietic) marrow [21]. The distinction between capillaries and sinusoids based solely on this characteristic should be done with caution, since fat cells are present in hematopoietic marrow [53] and sinusoids are observed in fatty marrow. The filtrate from the capillaries and sinusoids enter into the interstitial spaces of marrow and the porosities of bone. The porosities comprise of the Volkmann and Haversian canals, the lacunar-canalicular network of osteocytes and their dendritic processes, and the spaces within the mineral hydroxyapatite [56]. In mature animals, ~ 1/3rd of cortical bone contains fluid located in blood vessels (~ 6%), cells (~ 25%), and the interstitial space (~ 69%) [54]. In immature animals, the fluid spaces in cortical bone are larger [54].

Interstitial fluid is sourced by the marrow capillaries [57], which are supplied by bone and marrow arterioles and arteries. The nutrient arteries serve as bridges between the circulation of bone and that of the periphery, regulating blood flow and intramedullary pressure [58]. Recent investigations have revealed interesting findings in relation to newly discovered transcortical blood vessels, which are suspected to play the major role in blood supply to the skeleton [59]. In regard to flow, ~ 5–7% of cardiac output in male rats 2, 6, and 24 months of age went to the skeleton, which represented ~ 7–8% of the total body mass [60]. Blood flow to bone is on par with blood flow to some resting skeletal muscles [61] and, relative to cell mass, the skeleton receives a significant blood supply [54]. In regard to intramedullary pressure, recorded values have demonstrated high variability. For example, mean intramedullary pressures of ~ 16 mmHg were observed in C57BL/6 mice [62] vs. ~ 33 mmHg in adult New Zealand White rabbits [63]. The recorded variability no doubt reflects the diversity in the experimental protocols. Since intramedullary pressure is usually lower than blood pressure (i.e., a pressure differential), plasma can filtrate and allow for exchange of fluid between the vascular system, the interstitial space, and the lacunar-canalicular network [56].

Combined with the pressure differential between the vascular system and interstitial space, mechanical loading also drives the movement of interstitial fluid, allowing for the transport of nutrients and factors to and the removal of waste products from osteocytes embedded within bone [56]. Mechanical compression of bone and release of that compression expels and reuptakes interstitial fluid, respectively [64]. The porous nature of the skeleton permits the passage of interstitial fluid throughout bone [64] and shifts in interstitial fluid activate bone cells [56]. As a result, bone tissue is able to adapt to mechanical loading [65] via this indirect stimulus. As proof of concept, experimental conditions that eliminated or reduced mechanical loading, but altered bone intramedullary pressure and fluid flow, initiated bone formation [62, 66–69].

Bone adaptation to these alterations in interstitial fluid flow results from the creation of shear stress on bone cell surfaces, which stimulates bone anabolism or catabolism [56]. Mechanisms by which bone anabolism or catabolism occur are via release of shear stress-related factors such as nitric oxide and prostaglandins (e.g., PGE_2) from bone cells [70–73]. Nitric oxide participates in osteoblast differentiation [74, 75] and impairs osteoclast activity [76]. In addition, PGE_2 has been shown to inhibit bone loss with hind limb immobilization, such that trabecular bone was higher vs. age-matched controls [77]. In fact, vascular endothelial cells release a variety of factors (e.g., adenosine triphosphate, adenosine diphosphate, adenosine monophosphate, adenosine, prostacyclin, interleukin-11, insulin-like growth factor-1, endothelin-1, RANKL) that modulate bone cellular activity [78–87], indicating a regulatory function of bone blood vessels on bone in this regard. In conclusion, the delivery of blood flow to the skeleton via the bone vascular network ensures the fluidity of bone. As a result of pressure differentials created by the vascular system and mechanical loading, interstitial fluid flow throughout bone serves as the stimulus to augment or depress bone remodeling.

Reliance on the Bone Vascular Network for Egress and Ingress of Bone Marrow Cells to and from the Peripheral Circulation

Marrow, located in the diaphyseal shaft and the intratrabecular spaces of the metaphyses, is classified as either hematopoietic or fatty. The distinction between the two is minimal, however, since hematopoietic marrow contains fat cells [53]. In the adult skeleton, hematopoiesis occurs primarily in the marrow [88]. Hematopoiesis is the development and formation of all blood cells, which develop from a single precursor cell; i.e., the hematopoietic stem cell (HSC) [89]. HSCs have several unique properties that distinguish them from other cell types; e.g., (1) they can survive up to the entire lifespan of the organism, (2) they have self-replicative capacities, and (3) can proliferate broadly, producing all lineages of myeloid and lymphoid blood progenies [90] [89]. HSCs grow and mature on a lattice of non-hematopoietic stromal cells (e.g., fat cells, fibroblasts, endothelial cells, and macrophages), which assist in the development and differentiation of HSCs by providing a hematopoietic-inducing environment [89]. Thus, the infrastructure capable of hematopoietic cell renewal (i.e., a constant production of the various blood cells per unit volume of blood) is located within the marrow of the bones throughout the skeleton [91]. Marrow is therefore important for the integrity of the whole organism. Due to these lifelong duties, immense cell turnover rates occur, with ~500 billion cells being produced daily [91]. In states of pathology, hematopoiesis can occur in extramedullary tissues (i.e., the spleen, liver, and lymph nodes); however, this occurs at the cost of reduced efficiency [91].

In the 1970s, the HSC niche was first described as a three-dimensional space that housed HSCs and provided the regulatory environment for self-renewal and proliferation [92], which is critical for tissue homeostasis [93]. The HSCs located at the bone endosteum were referred to as the osteoblast niche, since HSCs adhere to bone-lining osteoblasts [94]. Early experimental evidence suggests that as progenitor cells (originating from the endosteal HSC pool) mature and differentiate, they migrate towards the marrow sinusoids [95–97]. The perivascular space surrounding the marrow sinusoids was coined the vascular niche. Thus, the osteoblast niche represented HSC quiescence, while the vascular niche represented HSC proliferation and mobilization [98]. Current evidence suggests that the osteoblast and vascular niches are both related to blood vessels and they have been coined the arteriolar-pericyte niche and the sinusoidal-megakaryocyte niche [99]. Both reside at the endosteal surface, maintaining the postulate that osteoblasts are still a component of the niche [99]. Further, subset niches have been demonstrated in the central marrow [100, 101], countering long-held beliefs of an endosteal preference.

The establishment of marrow hematopoiesis and osteogenesis is highly coordinated [102–104] and bone and marrow are linked by the vascular system [105]. During development, hematopoiesis and vascularization occur in tandem [93] and it is theorized that bone and marrow are organized according to the spatial distribution of blood vessels [38, 106, 107]. For example, there is a long-held belief that the preponderance of metabolically active marrow (i.e., hematopoietic) is in close proximity to the endosteal surface of bone [104], with a sparse amount of activity occurring in the central area of the marrow cavity [105]. However, recent data suggest that the majority of HSC are perivascular, located in highly vascularized regions at the endosteal surface [108, 109]. This corresponds to the spatial arrangement of the bone microcirculation, whereby a rich plexus of sinusoids line the bone endosteum [55], providing collection sites for mature blood cells and greater flow to areas of the marrow with higher metabolic rates. The endosteal sinusoids extend branches into the adjacent bone and also extend branches into the central marrow area, towards the central sinus [55]. Following suit, non-pathological blood flow follows a centrifugal direction; i.e., from the center of the marrow towards the endosteal surface [53, 55]. It has been demonstrated in the long bones of mice that arterial blood flows through the H-type capillaries, then the L-type sinusoids, and finally into the large central vein of the diaphysis for egress [110]. Further, blood velocity and shear stress are higher through the H-type capillaries vs. the L-type sinusoids [110]. Once blood enters the sinusoidal plexus at the endosteal

surface [55], it flows back towards the central sinus in a centripetal direction [55, 110]. Most recently, however, discovery of transcortical blood vessels suggests a large volume of blood leaves the skeleton via these routes [59]. Nevertheless, the amalgamation of the vascular, skeletal, and hematopoietic systems at the endosteal surface may modulate bone formation as well as hematopoiesis [88].

The marrow sinusoids, consisting of a single layer of endothelial cells with an incomplete basal membrane and discontinuous adventitial layer of perivascular cells [111], allow for the migration of mature blood cells into the peripheral circulation [55, 112–117]. In fact, passage of mature leukocytes and immature hematopoietic stem and progenitor cells in a murine model demonstrated access into the peripheral circulation exclusively via a sinusoidal route [118]. This occurs via a transendothelial route [114], with the formation of temporary pores in which the blood cells can pass via diapedesis [111]. The newly formed blood cells are collected into the sinusoids and the central sinus for release into the blood stream [91]. Vascular endothelial cells also play a more direct role in stimulating and regulating myelopoiesis (i.e., the production of marrow and cells) via manufacture of myeloid growth factors (i.e., colony-stimulating factors) when subjected to interleukin-1 [21]. Further, the vascular endothelium participates in the homing of cells to the marrow. Transplanted hematopoietic stem and progenitor cells in $Has3^{-/-}$ mice exhibited delayed transendothelial migration across the sinusoids in the femoral metaphysis in comparison to wild-type controls [119]. Has3 is the synthase that produces hyaluronic acid, which provides a scaffold of support and localizes, retains, and binds hematopoietic stem cells [120]. Thus, the function of marrow is reliant upon an intact microvascular system [121]; i.e., hematopoietic stem cells home to marrow and to other organs via the bone sinusoidal network [91, 122].

For blood cells incapable of accessing the peripheral circulation via diapedesis (i.e., red blood cells), growth pressure and pliability of their membranes are key factors. Red blood cell passage into the peripheral circulation relies upon alterations in intramedullary pressure induced by blood flow through bone [123]. The investigation of intramedullary pressure within bone has not been given proper attention in regard to its role in regulating the release of morphotic blood elements [123], but should be considered as a factor potentially contributing to the overall health of an organism. Red blood cell release into the circulation results from extravascular and vascular factors [123]. Within the rigid bone capsule, two forces opposed one another; i.e., the pressure generated by blood flowing through the sinusoids opposes the pressures generated by proliferating cells in the surrounding marrow parenchyma [91]. As proliferation pressure in the marrow parenchyma augments, it causes the nearby sinusoids to close [91]. Under circumstances that increase local blood flow, closed sinusoids can reopen [91]. At this time, mature cells that line the sinusoids are captured into the blood steam and carried into the peripheral circulation [91]. Thus, blood flow through the sinusoids is a key determinant of blood cell release from the marrow [117]. The sinusoids are in constant flux [55] and oscillations between the open or closed state permit a consistent exchange of parenchymal cells into the collecting sinuses [91]. In addition, recent finding demonstrated that neutrophil mobilization from the marrow can occur through the transcortical vessels [59]. Thus, the vascular system is at the forefront of the proper regulation of marrow function; i.e., it is essential for the mobilization and egress of blood cells (i.e., white and red blood cells and platelets) [95, 124, 125].

In total, vascular function is important for bone and marrow homeostasis as well as organismal health. Figure 1 summarizes how the bone and marrow microcirculation, via its regulation of blood flow, contributes to adequate nutrition of bone and marrow cells, supports hematopoiesis, and is requisite for bone development and repair. In addition, via the regulation of interstitial fluid flow and intramedullary pressure, the bone and marrow microcirculation provide factors that stimulate or depress bone cellular activity, allow for the transference of mechanical stimuli into chemical signals, and permit the collection and egress of mature blood cells into the peripheral circulation.

The Aging Blood Vessel

In association with age-related declines in bone mass, the cardiovascular system develops multiple pathologies. Recent studies have associated cardiovascular ailments (i.e., peripheral arterial disease, cardiovascular disease) and osteoporosis [126, 127]. More specifically, development of diseases in the bone vascular system have been reported [9, 10, 12, 13, 41], which have a more direct and profound influence on bone and marrow homeostasis. The end result of bone vascular pathology is reduced blood flow [4, 6] and marrow ischemia [128].

Vascular involvement in bone disease has been theorized since the early part of the twentieth century. In an article examining senile osteoporosis, Spencer, Hausinger, and Laszlo theorized that "a decline in blood supply due to degenerative vascular changes, impairment of capillary distribution and altered permeability of the capillary wall affecting the exchange of nutrients and waste products, may also be contributing factors" [129]. In the subsequent decades, many investigators followed along these lines of thinking and provided a multitude of experimental data in support [4, 6, 7, 10, 14–16, 53, 110, 128, 130–133]. Despite their efforts, investigations examining the vascular involvement of bone disease were limited by available technologies. While remaining a difficult subject to examine due to the necessity of penetrating bone to observe the vascular network, modern technologies have permitted more advanced visualization and investigative techniques. Over the years,

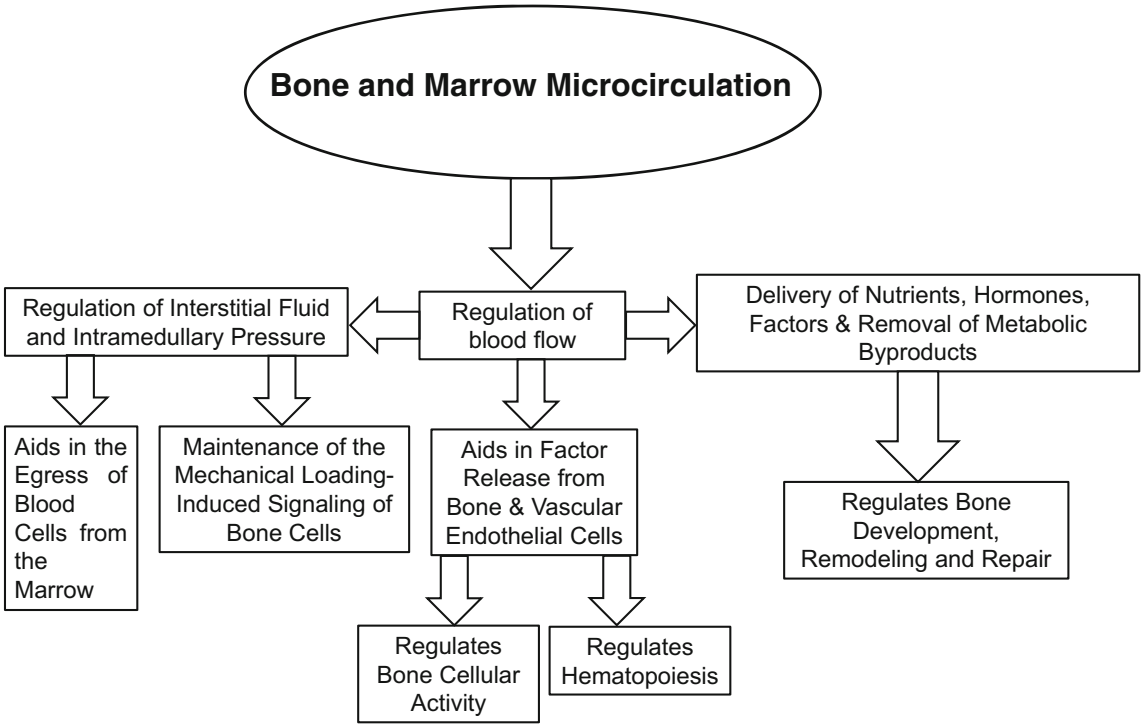

Fig. 1 The contribution of the microcirculation to bone and marrow homeostasis

experimental data has proven that blood delivery to bone with advancing age diminishes in both animal models [4, 6, 16, 110, 133] and humans [14, 15]. Various techniques (e.g., radiolabeled microspheres [4, 6, 16], magnetic resonance imaging [133], proton magnetic resonance spectroscopy [14, 15], intravital microscopy [110], and dynamic contrast-enhanced magnetic resonance [14, 15]) have been employed to examine this age-related phenomenon. Further, the observed decrements in bone blood flow have coincided with several vascular abnormalities (e.g., vascular rarefaction, reduced angiogenic capabilities, diminished vasodilator capacity, vascular calcification, arteriosclerosis, and atherosclerosis). Vascular rarefaction (i.e., a reduced number of blood vessels) has been reported in bone and marrow [9, 10, 128, 134]. Coupled with a reduced capacity to create new blood vessels, as demonstrated during fracture repair in elderly mice [135], once vascular rarefaction has occurred, restoration of an adequate vascular density is not easily achieved. Impaired angiogenesis may be reflective of the loss of H-type blood vessels recently discovered in a murine model and suspected to be highly associated with angiogenesis [41]. For example, H-type blood vessels were diminished in 70-week-old vs. 4-week-old mice, which corresponded with a loss of osteoprogenitor cells and bone mass in advanced age [41].

In addition to reduced vascular density and impaired angiogenesis, pathologies related to the function of blood vessels have also been observed. Similar to other tissue beds, bone blood vessels demonstrate age-related diminished vasodilator capacity. For example, decrements (27–55%) in endothelium-dependent vasodilation of the femoral principal nutrient artery (PNA) have been observed in 22–24-month-old vs. 4–6-month-old female and male rats [4–8]. In addition, arteriosclerosis and atherosclerosis have been observed in the bone vasculature [11–13], with arteriosclerosis being linked to diminished marrow arterial pressure and marrow ischemia [128]. Arteriosclerosis is suspected to occur ~ 10 years in advance of the same disease in blood vessels from other tissue beds [12] and atherosclerosis was observed in bone marrow blood vessels from human subjects, such that the cross-sectional lumen area was reduced by 18–26% in 50-year-old vs. 30-year-old individuals [13]. Interestingly, induction of atherosclerosis in rhesus monkeys fed a high-fat diet resulted in diminished skeletal blood flow, even though the lesions were observed in blood vessels outside of the skeleton [136].

A recent discovery demonstrated a pathology more severe than arteriosclerosis and atherosclerosis. For example, severe mineralization and calcification was observed on bone marrow blood vessels from the medullary cavity of young and old rats and human patients [9]. Morphologically, these vessels appear ossified and resembled bone, such that osteocyte lacunae and osteoid seams were visible on the abluminal surfaces [9]. Analysis with micro-CT demonstrated progressive ossification of the bone marrow vasculature as a function of advancing age in male rats [9]. The presumed conversion of blood vessels into bone (i.e., ossification) represents "microvascular dead space"; i.e., diminished vasodilation, vasoconstriction, and patency. To date, it is unknown which blood vessels (i.e., arteries, arterioles, capillaries, sinusoids, venules, and/or veins) undergo ossification. If the sinusoids suffer such

pathology, another functional consequence may include reduced egress of mature blood cells from the marrow.

The Aging Bone Marrow

Anemia is a common and underestimated condition in the elderly [137], can increase mortality rates [138], diminish physical performance, and attenuate activities of daily living [125, 139]. Anemia is a reduced red blood cell count and is defined by the World Health Organization as hemoglobin levels of ≤ 12 g/dL for women and ≤ 13 g/dL for men [137]. Mahlknecht and Kaiser reported age-related declines in hemoglobin and hematocrit for both genders [137]. The cases of anemia in the elderly can be attributed to several factors (e.g., chronic disease, infection, iron or vitamin B12 deficiency) [140, 141]. However, ~ 36% of cases in the elderly have an unknown origin [137, 140, 141] and are referred to as senile anemia [142]. In such instances, reduced blood counts are linked to reduced hematopoietic stem cells [143], progenitor cell differentiation anomalies [144], inability to mobilize progenitor cells [145], and attenuated responses to hormonal stimulation [146–149]. An impaired ability to generate and mobilize blood cells into the peripheral circulation would also include those of the lymphoid lineage. Thus, on a broader scale, pancytopenia is clinically diagnosed as a reduction in the number of white and red blood cells and platelets. Of particular note, some elderly individuals present with an attenuated host defense mechanisms and a diminished neutrophil response to infection [150]. While many factors contribute to reduce blood cell counts in the elderly, few researchers have examined a vascular etiology in relation to these symptoms. This review highlighted the importance of the marrow microcirculation in maintaining the HSC niches and for the mobilization and egress of blood cells. Further, this review demonstrated several age-related pathologies in the bone and marrow microcirculation that can have a dramatic impact on bone and marrow blood flow. Thus, the possibility exists that the etiologies contributing to senile anemia and/or pancytopenia may be partially related to vascular decline.

Hematopoietic marrow is progressively replaced by fatty marrow with advancing age, whereby for each decade of life, there is a 10% reduction in cellularity [151]. A study examining iliac crest biopsies of human subjects revealed an age-related diminution of the number of marrow sinusoids that corresponded with a reduced hematopoietic compartment and fat cell augmentation [10]. Clinically, osteoporosis and osteopenia in men were associated with augmented vertebral marrow fat vs. individuals with normal bone density [15] and marrow perfusion was reduced in the osteoporotic vs. osteopenic and control subjects [15]. Animal models have demonstrated similar trends, whereby declines in hematopoietic marrow coincided with augmented adiposity in the proximal tibia of 42-month-old vs. ≤ 6-month-old rabbits [152]. Accordingly, reduced marrow cellularity corresponded with diminished peripheral blood counts in individuals > 60 years of age [144, 153]. Additionally, arteriolar and capillary densities were reduced in 65–70-week-old vs. 4-week-old mouse tibiae and corresponded with diminished presence of perivascular mesenchymal cells and stromal cell factor [154]. Mesenchymal cells are linked to HSC regulation [155] and stromal cell factor participates in HSC maintenance and homing [156, 157]. Interestingly, experimental enhancement of vascular niche function (i.e., augmenting arterioles, capillaries, perivascular cells, stromal cell factor, and HSC number) in old mice tibiae could not restore the age-related declines of HSC function [154], which is suspected to result from abnormalities intrinsic to the aged HSC [158, 159].

Since marrow function is reliant upon an intact microvascular system [121], one has to speculate if the age-related changes in hematopoietic and fatty marrow are related to pathologies that would ultimately serve to reduce skeletal blood flow. In fact, blood flow to areas of high hematopoietic activity (i.e., marrow and trabecular bone) is augmented in comparison with areas of low hematopoietic activity (i.e., the cortical shell) [4, 6, 160]. Further, blood flow to bone and marrow is lower in advanced age [4, 6, 128], coinciding with reduced hematopoiesis and increased marrow adiposity [9, 10, 152]. Blood flow through the marrow sinusoids allows for the capture and egress of mature blood cells [91, 117, 122, 161]. Thus, one should rightly speculate that diseases such as arteriosclerosis [12], atherosclerosis [11, 13], and ossification [9] will impair this process. In fact, the relationship between atherosclerosis and senile anemia has been previously considered [13]. In addition, ossification of the marrow microcirculation may attenuate diapedesis of blood cells through the sinusoidal wall, if it contains bone and/or calcium deposits. Therefore, in addition to senile anemia, altered marrow morphology may have consequences on immune system ontogeny [162].

Aging and Fracture Repair

Fracture in the elderly coincides with elevated rates of morbidity and mortality [163] and age-related delays in fracture repair have been documented [164–166]. For example, during the inflammatory phase (day 3) following fracture of the tibia, periosteal cells differentiated into collagen X-expressing chondrocytes in juvenile (4 weeks of age) vs. middle-aged (6 months of age) and elderly (18 months of age) mice [166]. By day 5, juvenile mice displayed large volumes of cartilage in the tibial callus as opposed to smaller volumes of cartilage in the calluses of the middle-aged and elderly groups [166]. Further, at this time point, new bone and robust osteocalcin expression were observed in the periosteum of juvenile mice, while middle-aged and elderly mice demonstrated much

smaller quantities of both [166]. Vascularization at the fracture site follows the same sequential pattern. For example, while PECAM$^+$ blood vessels were observed in fracture calluses of juvenile (4 weeks of age), middle-aged (6 months of age), and elderly (18 months of age) 129J/B6 male mice, their presence was less numerous in the middle-aged and elderly groups [135]. This coincided with a higher surface density of blood vessels in the juveniles vs. the elderly [135]. Further, important regulators of angiogenesis (i.e., hypoxia-inducible factor-1α [HIF-1α] and VEGF) were also detected at early time points in juvenile calluses. For example, HIF-1α and VEGF transcripts were detected in the callus at 3 days following fracture in juveniles vs. 5 days following fracture in middle-aged and elderly mice [135]. This work demonstrates that the processes associated with vascularization during fracture repair are delayed or impaired by the aging process [135]. Further, bone volume and bone volume-to-total volume ratio in juvenile mice was higher than the middle-aged and elderly groups, suggesting an enhanced ability to form new bone in the youngest age group [166]. When assessed as a function of age and time, bone volume was higher in juvenile vs. middle-aged and elderly mice, indicating delayed healing in the older groups [166]. While many factors contribute to bone healing following fracture, the connection of vascular density with the appearance of new bone formation is noteworthy.

The Clinical Relevance of the Bone Vascular Network

This review has thus far highlighted the various physiological roles of the bone vascular network for bone and marrow function. While this review has focused on the age-related decrements in these three systems (i.e., vascular, bone, and marrow), we must recognize that advancing age is often associated with at least one morbidity and additional comorbidities. These morbidities and comorbidities often present with vascular challenges similar to those observed in advanced age. For example, declines in vasodilator (i.e., both endothelium-dependent and endothelium-independent) capacity of the rat femoral PNA were observed in a long-term (i.e., 20 weeks) type 2 diabetes mellitus model [167]. Type 2 diabetic rats also had elevated vasoconstriction to norepinephrine, enhanced myogenic vasoconstriction, and reduced mechanical distensibility vs. controls [167]. These vascular alterations corresponded with diminished bone and marrow blood flow [167] and decrements in various bone parameters (i.e., bone mineral density and strength) at several skeletal sites [168]. In addition to increasing the stiffness of the PNA, type 2 diabetes mellitus led to a more pro-vasoconstrictor phenotype of the vessel [167].

Loss of estrogen also alters the bone vascular network. While there is data demonstrating augmented bone blood flow, bone loss, and reduced bone mineral density and ash weight following ovariectomy and orchidectomy in young rats [169–171], more recent publications have documented the opposite. Estrogen-associated bone loss [7, 133] corresponded with diminished vasodilator capacity of bone arteries [7], enhanced vasoconstrictor capacity of bone arteries [172], reductions in erythropoietic marrow [133], vascular rarefaction [173], and declines in bone perfusion [133, 173]. Interestingly, strong, positive correlations existed between peak vasodilator capacity of bone arteries and bone volume, while poor associations existed between plasma estrogen and bone volume in animals with low circulating estrogen (i.e., old control and young and old ovariectomized) [7]. These data indicate that vascular function is a better predictor of bone loss than estrogen status [7].

The vascular alterations associated with hind limb unloading (i.e., a ground-based rodent model used to simulate bed rest, physical inactivity, and microgravity) drums home the clinical relevance associated with altered bone vascular function and structure in advanced age or morbidity. Following 2 weeks of hind limb unloading in 6-month-old male Fischer-344 rats, vasoconstriction to phenylephrine, an α1-receptor adrenergic agonist, was reduced in the femoral PNA [174]. Likewise, flow- and acetylcholine-mediated vasodilation (i.e., both endothelium-related mechanisms) were impaired in PNAs from hind limb unloaded animals [174]. In addition to the vasomotor changes, hind limb unloading structurally remodeled the PNA such that the intraluminal diameter (i.e., 146 ± 7 μm vs. 177 ± 10 μm, respectively) and medial wall thickness (i.e., 16 ± 2 μm vs. 22 ± 2 μm, respectively) were diminished in comparison to control rats [175]. Trabecular bone mineral density in the femur was also reduced [174]. The structural remodeling of the PNA resulted in an impaired ability to deliver blood [175]. Thus, 2 weeks of hind limb unloading remodeled the PNA and impaired its vasomotor function in such a way as to diminish its effectiveness at delivering blood to the femur upon re-initiation of weight-bearing activities [174, 175]. This set of experiments illustrates how alterations in vasomotor function or structure of bone arteries impact bone blood flow.

From a clinical perspective, the age- and/or disease-related alterations in the microcirculation of bone and marrow should not be overlooked, as they contribute to the declines in skeletal perfusion and presumably hinder the clinical treatment of bone and marrow. Singularly, one of these vascular dysfunctions (i.e., diminished vasodilator or enhanced vasoconstrictor activity, vascular rarefaction, arteriosclerosis, atherosclerosis, and ossification) could serve to diminish bone and marrow blood flow, initiating ischemia and affecting interstitial fluid flow and pressure, bone remodeling, fracture repair, and the egress of cell from the marrow. Suffering from more than one of these vascular pathologies, which is most likely the clinical scenario, could further exacerbate the ischemia and the

decrements in bone and marrow blood flow. Clinically speaking, it may be prudent to consider the treatment of bone or marrow pathology as binary; i.e., the treatment of either of these organ systems should be coordinated with the treatment of an aged and/or diseased vascular system. Experimental and/or clinical data in regard to such a strategy, to date, is lacking. However, experimental evidence related to intermittent parathyroid hormone (PTH) administration may provide support.

Intermittent PTH administration is bone anabolic and augments bone mass. Thus, from a historical perspective, the efficacy of PTH treatment has been attributed to its effects on bone cellular activity. Recent data, however, illustrate its impact on bone vascular function and morphology. Intermittent PTH administration enhanced endothelium-dependent vasodilator function in young and old bone blood vessels [8, 176], augmented bone vascular density [173], redistributed the smallest bone blood vessels closer to bone-forming sites [177], and increased skeletal perfusion [103, 173]. All of these modifications serve to aid in the regulation of bone cellular activity and promote an environment supportive of bone formation. A note of caution, however, as recent evidence suggests a potential exacerbation of bone marrow blood vessel ossification with intermittent PTH administration [178]. Interestingly, 2 weeks of intermittent PTH administration improved the age-related decrements in aortic function in old rats [179]. While prescribed to augment bone mass, the totality of these findings demonstrate an unintended benefit of this treatment on bone blood vessels and overall vascular function.

To date, diagnosing bone vascular dysfunction in the clinical setting is difficult, if not impossible. Thus, the clinical treatment of bone vascular pathology without a clear diagnosis is not recommended. However, non-pharmacological remedies are available. Treadmill exercise training in young and old rodents resulted in increased vasodilator capacity of the femoral PNA [6], augmented blood flow to the femur [6], enhanced bone angiogenesis [180], and elicited exercise hyperemia to more bone regions [181] vs. sedentary controls. These vascular enhancements coincided with augmented bone volume [6, 180] and mineral density [180], except when rats were administered an anti-angiogenic agent which prevented bone angiogenesis and, thus, the changes in bone volume [180]. While clinical and experimental data may be lacking as to the effects of various medications on bone vascular function, a prescription of exercise training or physical activity to patients suffering from bone and marrow ailments may enhance or restore function in the bone and marrow microcirculation, particularly if the individuals are of advanced age.

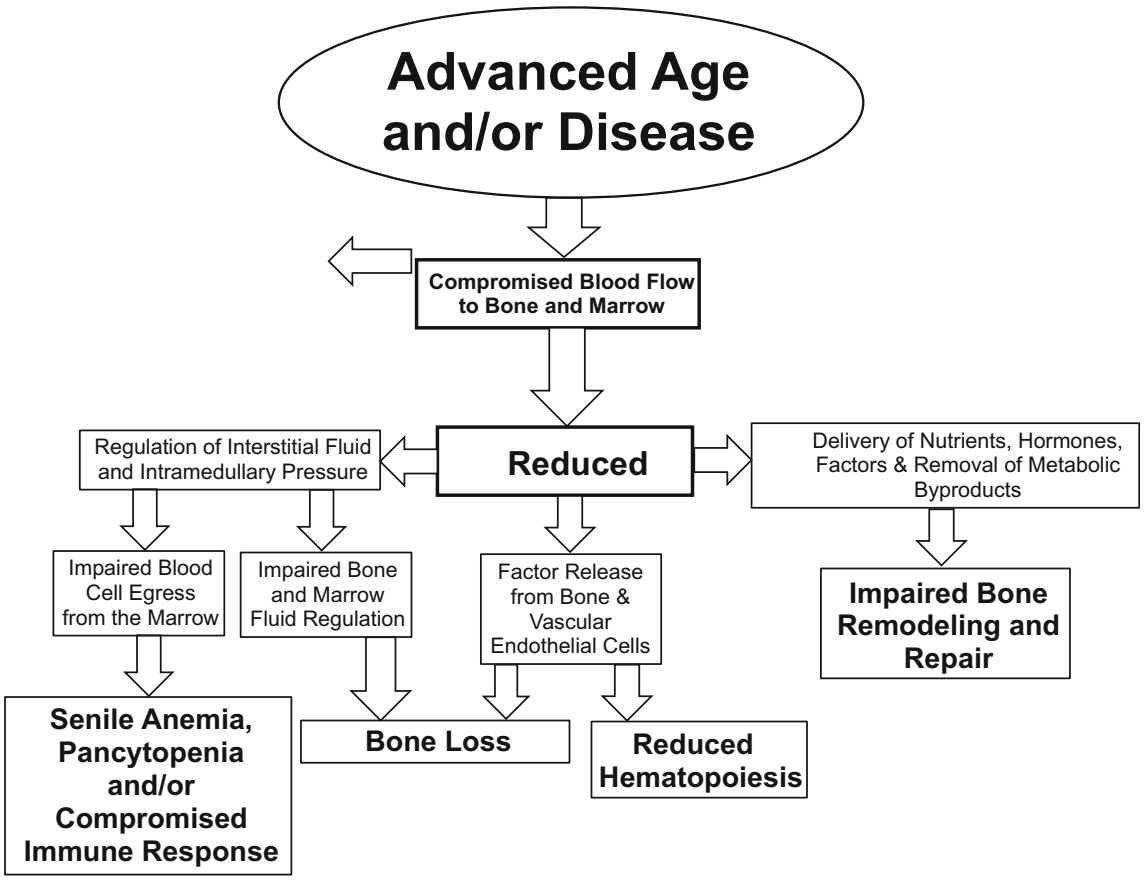

Fig. 2 Age- and/or disease-related bone vascular pathologies and the potential consequences on bone and marrow homeostasis

Decreased Efficacy of Bone-Targeted Therapies in the Setting of a Compromised Vascular Network

To date, there are no studies examining the effects of bone vascular dysfunction on drug delivery to bone and marrow. Further, there is no experimental evidence indicating a decreased efficacy of bone-targeted therapy as a function of advance age and/or disease. However, "the absence of evidence is not evidence of absence." Given the various physiological roles of the bone vascular network in supplying blood flow, removing waste products, regulating bone interstitial pressure and fluid flow, and mobilizing marrow cells for distribution into the peripheral circulation, it stands to reasons that compromises in this organ system would influence the ability to deliver drugs to bone and marrow. Perhaps it is time as a scientific and medical community to begin addressing these questions. At the extreme end of the clinical spectrum, steroid-induced osteonecrosis of the femoral head is associated with ischemia; i.e., diminish blood flow resulting from fat emboli or from hypertrophic adipocytes, which occlude or compress, respectively, the microcirculation [182–184]. To lesser extremes, circumstantial evidences provide associations between the various vascular pathologies and declines in bone health. For example, a vasoconstrictor phenotype of bone arteries in type 2 diabetes mellitus [7], age-, and/or disease-related diminished vasodilator capacity of bone blood vessels [4–8, 133], the presence of arteriosclerosis [12], atherosclerosis [11, 13] and ossification [9] in bone blood vessels, vascular rarefaction [9, 10, 41, 59, 128, 134], and reduced angiogenic capability [135] coincide with reduced skeletal blood flow [4, 6, 14–16, 110, 133, 167] and marrow ischemia [128]. Thus, in light of these connections, it may prove beneficial to begin clinical exploration as to whether an aging and/or diseased vascular system influences drug delivery or the efficacy of treating of bone and marrow pathology.

Conclusion

The vascular supply is essential for the maintenance of health and longevity of all tissues. In addition, the bone vascular network is vital for bone development (i.e., intramembranous and endochondral ossification), bone homeostasis (i.e., bone remodeling), and fracture repair. The regulation of bone blood flow by the nutrient arteries and arterioles allows for the fluidic nature of bone, which is essential for shear-mediated release of factors from vascular endothelial and bone cells, and for the transduction of mechanical stimuli into chemical signals. Finally, the mobilization and capture of mature blood cells depend upon the ability of the cells to penetrate the vascular wall, are reliant upon blood flow through the sinusoidal network, and are contingent upon pressure gradients between the sinusoids and the interstitial space. Due to these responsibilities, age- and/or disease-related vascular decline can have profound influences on bone and marrow homeostasis, as outlined in Fig. 2. The pathological consequences may include reduced bone and marrow blood flow, attenuated nutrient delivery and waste removal, impaired hematopoiesis, bone remodeling, and fracture healing, dysregulation of interstitial fluid flow and intramedullary pressure, impaired transference of mechanically-induced signals into bone cellular activity, and diminished egress of mature blood cells. The clinical manifestations of these abnormalities may present as bone loss, diminished fracture healing, increased fracture risk, senile anemia, and/or a compromised immune system. From a clinical standpoint, medications and treatments prescribed to alleviate these medical conditions may have reduced or no efficacy if relying upon a bone and marrow microcirculation with impaired or obstructed patency. These may be important clinical inquires to address to determine whether the efficacy of bone and marrow treatment is enhanced via the coinciding treatment of bone vascular dysfunction.

Compliance with Ethical Standards

Conflict of Interest The author declares that she has no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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