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Polycythemia is associated with bone loss and reduced osteoblast activity in mice

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Abstract

Summary—Increased fragility has been described in humans with polycythemia vera (PV). Herein, we describe an osteoporotic phenotype associated with decreased osteoblast activity in a mouse model of PV and another mouse of polycythemia and elevated circulating erythropoietin (EPO). Our results are important for patients with PV or those treated with recombinant EPO (rEPO).

Introduction—PV and other myeloproliferative syndromes have been recently associated with an increased risk for fractures. However, the presence of osteoporosis in these patients has not been well documented. EPO, a hormone primarily known to stimulate erythropoiesis, has been shown recently to regulate bone homeostasis in mice. The aim of this study was to examine the bone phenotype of a mouse model of PV and compare it to that of animals with polycythemia caused by elevated circulating EPO.

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Compliance with Ethical Standards

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed. All procedures performed were in accordance with the ethical standards of Weill Cornell Medical College at which the studies were conducted. All studies were approved by the Institutional Animal Care and Use Committees of Weill Cornell Medical College.

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

Conflicts of interest Dr. Rivella is a consultant for Novartis Pharmaceuticals, Isis Pharmaceuticals, Medgenics Pharmaceuticals, and Bayer Healthcare.

Paraskevi-Rea Oikonomidou, Carla Casu, Zhiwei Yang, Bart Crielaard, Jae-Hyuck Shim, and Maria G. Vogiatzi declare that they have no conflict of interest.

Methods—Bone mass and remodeling were evaluated by micro-computed tomography and histomorphometry. The JAK2^{V617F} knock-in mouse, a model of human PV, manifests polycythemia and low circulating EPO levels. Results from this mouse were compared to wild type (wt) controls and the tg6 transgenic mouse that shows polycythemia caused by increased constitutive expression of EPO.

Results—Compared to wt, both JAK2^{V617F} and tg6 mice had a decrease in trabecular bone mass. Tg6 mice showed an additional modest decrease in cortical thickness and cortical bone volume per tissue volume ($P<0.01$) suggesting a more severe bone phenotype than JAK2^{V617F}. Decreased osteoblast numbers and bone formation along with normal osteoclast numbers and activity were found in both mice.

Conclusions—This study indicates that PV is associated with low bone mass and decreased osteoblast activity in mice. Our results support future studies of osteoporosis in affected humans. Polycythemia caused by chronically elevated circulating EPO also results in bone loss, and implications on patients treated with rEPO should be evaluated.

Keywords

Erythropoietin; JAK2^{V617F}; Osteoblasts; Polycythemia; Tg6 mice

Introduction

Recent human and animal studies document an interaction between erythropoiesis and bone mass [1–6]. Humans with chronic hemolytic anemia such as thalassemias suffer from severe osteoporosis and fractures [7, 8]. Low bone mass has been described in individuals with sickle cell anemia [9]. In animals, a decrease in bone mass was observed after bleeding, phenylhydrazine-induced hemolysis, or inflammation induced anemia [10]. Beyond anemias, a recent report found increased fragility in patients suffering from disorders characterized by hematopoietic expansion, such as polycythemia vera (PV) and other chronic myeloproliferating syndromes [11]. However, the presence of osteoporosis in these patients has not yet systematically been studied. In mice, changes in bone mass were observed in association with a moderate increase in hematocrit after exogenous erythropoietin (EPO) administration [12]. These findings indicate an effect of erythroid expansion on bone homeostasis.

To better understand changes in bone homeostasis and remodeling with polycythemia, we studied a mouse model of PV. PV is characterized by accelerated clonal proliferation of hematopoietic progenitors leading primarily to an uncontrolled red blood cell production and absolute red blood cell mass expansion [13]. As expected, EPO secretion, which is the primary hormone responsible for red blood cell production, is suppressed in this disease [14, 15]. A somatic point mutation (V617F) in the Janus kinase 2 (JAK2) is found in the majority of the patients with PV [16, 17]. JAK2 plays an important role in signaling through the EPO receptor (EPOR), and the JAK2^{V617F} mutation leads to constitutional activation of EPOR signaling and EPO independent proliferation and differentiation of erythroid precursors to mature red blood cells [18]. A conditional knock-in mouse model with the JAK2^{V617F}

mutation has been described to recapitulate the hematological phenotype of PV [19]. However, bone phenotypes in this mouse have not been studied.

Serum EPO concentrations are suppressed in a JAK2^{V617F} knock-in mouse. EPO is a hormone that has attracted a lot of attention over the last decade for its potential, multiple functions beyond erythropoiesis. Specifically, recent research findings indicate that EPO may have additional anti-inflammatory, cardioprotective, and neuroprotective effects [20–26]. Among them, a possible effect of EPO on bone metabolism has been suggested by a number of animal studies. Indeed, systemic or local delivery of EPO at a fracture site in animals has shown positive effects in bone repair, cell proliferation, and angiogenesis [27–29]. Furthermore, systemic EPO administration in wild type (wt) mice resulted in an increase in hemoglobin (Hb) concentrations and changes in bone mass. However the results on the skeleton are controversial. One group of investigators showed a positive effect on bone mass and bone formation in newborn and older animals treated with EPO [12] while two other groups observed that exogenous EPO administration resulted in bone loss caused by increased bone turnover [30, 31]. The debate extends on the potential mechanisms of EPO action in vivo and whether EPO has direct humoral effects on bone homeostasis. The latter include a direct EPO effect on mesenchymal cell differentiation and EPOR signaling on hematopoietic stem cells [12]. Regardless, several in vitro studies suggest that EPO can increase osteogenesis through various pathways and raise the possibility of EPO having bone anabolic effects [32, 33].

To gain insight on the effects of EPO on the skeleton, we studied bone phenotypes in a JAK2^{V617F} knock-in mouse where serum EPO concentrations are suppressed and in an additional mouse model, named tg6, which is characterized by polycythemia caused by increased circulating EPO concentrations. This transgenic animal constitutively overexpresses EPO in an oxygen-independent manner [34, 35]. This mouse has a 10–12-fold elevation in EPO plasma levels [34] resulting in erythrocytosis [36] along with a decrease in bone mass [31]. Herein, we compared the bone phenotype of these two mice to determine the potential effects of EPO on the skeleton beyond polycythemia.

Methods

Animals

Animals were generously provided by Dr. Gassmann's laboratory [34]. The mice were maintained and bred at the Weill Cornell Medical College animal facility. Transgenic mice from the TgN(PDGFBEP0)321ZbZ mouse line, termed tg6, as well as JAK2^{V617F} knock-in mice [19] were used. Tg6 mice carry the human EPO cDNA expressed under the platelet-derived growth factor (PDGF) B chain promoter, which drives EPO expression in multiple tissues including hematopoietic cells, neuronal cells, and lung [35]. The mice manifest polycythemia with hematocrit values as high as 85 % and a $\times 10$ -fold rise in plasma EPO levels [34]. For this study, hemizygous tg6 males were bred with wild type (wt) C57BL/6 females to generate hemizygous and wt littermates, the latter being used as controls. The JAK2^{V617F} mouse is floxed, and for this study, it was crossed with animals expressing Cre recombinase in hematopoietic tissues under the control of the Vav regulatory element to generate double transgenic mice that carry a JAK2^{V617F} activating mutation only in the

hematopoietic cells (Vav-Cre/JAK2^{V617F/+}). The mice have an increase in hematocrit concentration similar to tg6 mice and represent an animal model of PV. Suppressed circulating EPO levels have been described in this model.

A Decreased number of littermates due to unexplained repeated pregnancy loss and decreased survival rate have been described in both tg6 and Vav-Cre/JAK2^{V617F/+} mouse models [19, 37]. To overcome this problem, and since these animals are known to be serially transplanted [19], we performed bone marrow (BM) transplantation into lethally irradiated wt mice. Two-month-old male wt C57BL/6 recipient mice were irradiated with 9.5 Gy (single dose) and transplanted by tail injection into the lateral veins with $2-3 \times 10^6$ donor BM cells obtained from tg6, Vav-Cre/JAK2^{V617F/+}, or wt mice on the day of the irradiation. The generated wt, tg6, and Vav-Cre/JAK2^{V617F/+} mice are referred in this paper as wt, tg6, and Vav-Cre/JAK2^{V617F/+}, respectively, and were analyzed 10 weeks after transplantation at the age of 4.5 months old. All studies were approved by the Institutional Animal Care and Use Committees of Weill Cornell Medical College.

Micro-computed tomography

Densitometry and morphometry micro-computed tomography (micro-CT) analysis was performed on cortical bone from mid-diaphyses of femurs and trabecular bone from distal femurs. The bones were scanned in their entirety by micro-CT 35 scanner (Scanco Medical, Bassersdorf, Switzerland). An isotropic 7- μ m voxel size was used in all the mice; 55 KVP were used in all scans. The reconstruction of 3D volumes from the 2D projections into CT volume data, densitometric analysis of the 3D volumes, and morphometric analysis was performed with image processing language (IPL 6.0) by Scanco (Scanco Medical, Bassersdorf, Switzerland).

Bone morphometric variables of cortical bone regions included bone area and thickness. The morphometric parameters defining trabecular bone included bone volume fraction (BV/TV), trabecular thickness (Tb.Th.), trabecular number (Tb.N.), and trabecular separation (Tb.Sp.).

Histology and histomorphometry

All studies were performed at the Yale Orthopaedic Histology and Histomorphometry laboratory according to previously established protocols [38]. In brief, the bones were stripped of soft tissues and fixed in 70% ethanol at 4 °C. After 24–48 h of fixation, the bones were dehydrated and cleared on a tissue processor then immersed in Acrylosin Infiltrate (Dorn and Hart, Villa Park, IL). Infiltration was carried out at 4 °C in a vacuum chamber for 48 h. Prepolymerized bases were prepared using 3 ml of embedding mix: 100 ml of Acrylosin Soft (Dorn and Hart, Villa Park, IL) with 0.26 g of Perkadox-16, in a glass scintillation vial placed in a 37 °C oven for 48 h to polymerize. Infiltrated samples were placed on the prepolymerized base and covered with 10 ml of embedding mix. The vial was then placed in a water bath in a 37 °C oven for 48 h to complete polymerization.

Once polymerized, vials were removed from the oven, placed in a –20 °C freezer for 15–20 min, and the glass vial was removed. Blocks were then trimmed on a band saw and sanded to area of interest on a Buehler MetaServ (Lake Bluff, IL) grinder. Five- μ m-thick sections were obtained using a Leica 2265 Microtome (Buffalo Grove, IL) and a 160-mm D-profile

tungsten carbide knife (Dorn and Hart, Villa Park, IL), placed on chromium-alum gelatin-coated slides and dried overnight in an oven at 37 °C. Slides were then deplastified with Cellosolve (Fisher), rehydrated through graded ethanols to distilled water, and stained with Toluidine Blue O (Fisher), pH 4.3. Unstained sections were coverslipped with a UV inert mountant for the visualization of fluorescent markers of mineralization.

Microscopic analysis of static and dynamic parameters was performed using an Olympus microscope interfaced with the Osteomeasure system software and hardware (Osteometrics, Atlanta, GA). The software calculations of various parameters are based on formulas defined by Parfitt, et al. [39]. All static parameters were measured on trabecular bone beginning immediately under the growth plate excluding endosteal surfaces. Field size for static parameters was 350 μm and the area measured under the growth plate extended 700 μm under the growth plate where most of the trabecular bone is located. For dynamic histomorphometry, animals were injected with calcein 7 and 2 days prior to sacrifice. Dynamic parameters of bone formation were read similarly using a Nikon scope equipped with epifluorescence to visualize pulse-labeled calcein on the trabecular surfaces.

Osteoclast number/bone perimeter (n/ μm) and osteoclast surface/bone surface (%) were evaluated after staining the sections for tartrate-resistant acid phosphatase (TRACP).

Hematological analysis and assays

Blood samples were collected by retro-orbital puncture under anesthesia. Hematological parameters were measured on an Advia 120 Hematology System (Bayer, Tarrytown, NY). Serum levels of erythropoietin were detected by ELISA according to the provided protocols (Human Erythropoietin Quantikine IVD ELISA kit, Mouse Erythropoietin Quantikine ELISA kit, R&D Systems). Serum levels of bone-related degradation products from C-terminal telopeptides of type I collagen (CTX-I) were detected by EIA according to the provided protocols (RatLaps (CTX-I), IDS).

Statistical analysis

Results are presented as means $\pm$ SD. The significance of results was analyzed using the analysis of variance (ANOVA) and unpaired two-tailed Student *t* test. The level of significance for all statistical tests was **P*<0.05, ***P*<0.01. Data were analyzed by GraphPad Prism 6.0 for Windows (GraphPad Software Inc., San Diego, CA, USA).

Results

Tg6 and Vav-Cre/JAK2^{V617F/+} mice manifest severe polycythemia

Ten weeks after BM transplantation Vav-Cre/JAK2^{V617F/+}, tg6, and wt mice (10 mice/group; all males) were all evaluated at the age of 4.5 months to examine the phenotype of polycythemia. Both tg6 and Vav-Cre/JAK2^{V617F/+} mice exhibited severe polycythemia and had similar hematopoietic values (hemoglobin levels of wt, 13.68 $\pm$ 1.39 g/dl; Vav-Cre/JAK2^{V617F/+}, 18.72 $\pm$ 1.68 g/dl; tg6, 20.51 $\pm$ 1.14 g/dl) (*P*<0.01, wt vs. Vav-Cre/JAK2^{V617F/+} and wt vs. tg6; *P*=NS Vav-Cre/JAK2^{V617F/+} vs. tg6). Increased erythropoietic activity and extramedullary hematopoiesis was also indicated by enlarged spleens in both tg6 and Vav-

Cre/JAK2^{V617F/+} mice (data not shown). As previously reported, tg6 mice showed elevated serum EPO levels compared to wt mice [34] whereas serum EPO levels were reduced in Vav-Cre/JAK2^{V617F/+} mice compared with wt mice, in accordance to the human polycythemia vera phenotype [15].

Tg6 and Vav-Cre/JAK2^{V617F/+} mice exhibit osteopenia

Micro-CT analysis of trabecular bone at distal femur revealed an osteoporotic phenotype in both tg6 and Vav-cre/JAK2^{V617F/+} mice (Fig. 1). Specifically, we observed a decrease in bone volume ratio (BV/TV) and trabecular number (Tb.N.) with a concomitant increase in trabecular separation (Tb.Sp.) in both animal models compared to wt controls (Fig. 2a–d). Thus, this data demonstrates a similar degree of trabecular bone loss in the two mouse models, suggesting that polycythemia results in a significant loss of trabecular bone in mice.

In contrast to trabecular bone loss observed in both mouse models, a decrease in cortical thickness (C.Th) and cortical bone volume per tissue volume (C.BV/TV) was detected only in the tg6 mice (C.Th., 0.18 ± 0.01 vs. 0.16 ± 0.01 mm, $P = 0.004$; C.BV/TV, 0.43 ± 0.02 vs. 0.39 ± 0.01 , $P = 0.0005$, wt vs. tg6, mean $\pm$ SD), but not in Vav-cre/JAK2^{V617F/+} mice (Fig. 2e–f). Taken together, our results demonstrate a significant loss of trabecular bone in both tg6 and Vav-cre/JAK2^{V617F/+} mice compared to wt controls while cortical bone mass was decreased only in tg6 mice.

Bone formation is decreased in tg6 and Vav-Cre/JAK2^{V617F/+} mice

In order to determine alterations in bone remodeling leading to bone loss in the tg6 and Vav-cre/JAK2^{V617F/+} animals we performed dynamic histomorphometry analysis. In agreement with the micro-CT results, we observed decreased bone volume ratio and trabecular number in both mouse models compared to wt animals (Fig. 3b–c). Osteoblast numbers (NOb/TAR) are significantly decreased in both tg6 and Vav-Cre/JAK2^{V617F/+} mice (Fig. 3d) (159.4 ± 46 vs. 93.6 ± 20 vs. 55.7 ± 22 NOb/mm², wt vs. Vav-Cre/JAK2^{V617F/+} vs. tg6, $P < 0.001$) whereas osteoid volume per bone volume (OV/BV) and osteoid thickness (O.Th) were not affected by polycythemia (Fig. 3e–f). Additionally, osteoblast surface per bone surface was decreased (Obs/BS), in tg6 mice and Vav-Cre/JAK2^{V617F/+} mice even though significance was not established in Vav-Cre/JAK2^{V617F/+} mice (Fig. 3g). To further evaluate osteoblast function, we assessed bone formation rate per tissue volume (BFR/TV) by in vivo double fluorescent calcein labeling (Fig. 3h). Accompanied with a decrease in osteoblast number, BFR/TV was decreased by 35.6 % in the Vav-Cre/JAK2^{V617F/+} mice and 36.8 % in tg6 mice compared to wt mice. Thus, these results suggest that polycythemia contributes to reduction of osteoblast number and activity.

Osteoclasts are normal in tg6 and Vav-Cre/JAK2^{V617F/+} mice

To investigate if bone resorption is affected by polycythemia, we further analyzed osteoclast numbers and function in tg6 and Vav-Cre/JAK2^{V617F/+} mice. Staining for tartrate-resistant acid phosphatase (TRAP) in histomorphometry analysis revealed that numbers of osteoclasts (NOc/BPm) or osteoclast surface per bone surface ratio (OcS/BS) are all normal in these mice (Fig. 4). Additionally, the serum levels of carboxy-terminal collagen crosslink (CTX) in tg6 and Vav-Cre/JAK2^{V617F/+} mice were comparable to those in wt mice (wt, 17.97 ± 3.18 ;

Vav-Cre/JAK2^{V617F/+}, 18.26±2.66; tg6, 21.77±5.95 ng/ml), indicating that osteoclast activity is unaltered by polycythemia (data not shown).

Discussion

This study reports on the bone phenotype of two mouse models of polycythemia, tg6 and Vav-Cre/JAK2^{V617F}. Both mice have markedly elevated hemoglobin concentrations, in the range of 18.5 to 20 g/dl. Using micro-CT, we observed similar bone loss in both mice suggesting that chronic polycythemia affects bone remodeling and can be a risk factor for osteoporosis. In accordance to our animal data, a clinical study by Farmer et al. showed that the risk of femoral fractures is increased by 4.74 % in patients 5 years after diagnosis of polycythemia vera compared to the general population [11]. Increased fragility was also observed among patients with other myeloproliferative syndromes, which are also characterized by hematopoietic expansion. Taken together, these findings point to the need for further studies to better understand the risk of osteoporosis in patients affected with these disorders.

Our histology and dynamic histomorphometry analyses revealed a significant decrease in osteoblast numbers and bone formation in both animals. Impaired osteogenesis was recently described in the tg6 mouse by other investigators and was likewise reported in animal models of thalassemia [31, 40–42]. In this disorder, a defective hemoglobin molecule leads to continuous erythroid destruction and anemia, which then causes a rise in circulating EPO and a subsequent expansion of defective erythroids, in a vicious circle of “ineffective erythropoiesis”. Decreased bone formation was also observed in humans with the disease [43, 44]. Therefore, we suggest that chronic expansion of the erythroid lineage can affect osteoblast numbers and lead to impaired bone formation.

The exact mechanism(s) by which erythropoiesis may regulate osteogenesis remain a topic of debate. Several studies support a central role of EPO in this process including a direct positive effect of EPO on osteogenesis. Specifically, Taichman's group observed that hematopoiesis alters mesenchymal stem cell (MSC) differentiation and increases osteogenesis through mechanisms that involve MSC interactions with hematopoietic stem cells (HSC), EPOR-JAK2 signaling at HSC, and direct EPOR signaling at MSC [1, 4, 12, 32]. Animal studies of EPO administration by the same group as well as studies of EPO given for bone regeneration support an anabolic effect of EPO on the skeleton [12, 29]. Subsequent studies in mice by two independent groups failed to replicate these findings and documented bone loss associated with EPO administration and high circulating EPO [30, 31]. Finally, the controversy extends in the area of osteoclastogenesis. While some investigators observed a direct EPO effect on osteoclast precursors, others failed to confirm these findings [1, 30, 31]. The current study does not support an effect of EPOR signaling on the osteoclast, as bone resorption was not altered in the mice. Differences in study design and methodologies are likely to explain some of these discrepancies.

The mechanism that leads to hematopoietic expansion varies in the two animals of this study. Specifically, in the tg6 animal, hematopoietic expansion is driven by EPO overexpression, whereas the constitutional activation of the EPOR signaling in

hematopoietic tissues leads to polycythemia irrespective of circulating serum EPO in the Vav-Cre/JAK2^{V617F} mouse. We describe that the two mice have trabecular bone loss of similar severity. However, the cortical bone was modestly affected only in the tg6 mouse and not in the Vav-Cre/JAK2^{V617F}, suggesting a greater impact on bone mass in this model. In support of this statement, our histomorphometry data also suggests that osteoblast activity may be more impaired in the tg6 mouse. Therefore, one may argue that there may be an additional negative effect on bone homeostasis mediated by the chronically elevated circulating EPO of the tg6 mouse. These findings need confirmation with further studies. Recombinant EPO is widely prescribed in clinical practice today, especially among patients with end-stage renal disease. Understanding the potential effects of EPO on the skeleton would be beneficial for these patients. In addition, EPO is being used illegally as a performance-enhancing drug in athletes, and bone loss can be a yet non-appreciated complication of such treatment. Finally, the mice used in this study were generated after BM transplantation to overcome difficulties from decreased survival rates of the transgenic animals. It is possible that a failure to detect greater changes in the cortical bone is related to the BM transplantation that was performed at the age of 2 months when a considerable part of bone growth has already been achieved [40, 45]. While our micro-CT data of the tg6 mouse are in agreement with a recent report by Hiram-Bab et al., yet we did not detect an increase in bone resorption as these investigators [31]. This difference is unlikely to be related to an effect of BM transplantation, as we have previously reported that BM irradiation and transplantation does not suppress osteoclast activity and function in wt mice [40].

In summary, the present study demonstrates that polycythemia is associated with bone loss and decreased osteoblast function in two mouse models. The Vav-Cre/JAK2^{V617F} mouse, in particular, is a mouse model of polycythemia vera, and bone loss in this animal provides rationale for additional clinical studies to understand the prevalence and severity of osteoporosis in patients affected with this disorder. Our findings do not support an anabolic effect of EPO on the skeleton. Whether chronically elevated circulating EPO can have an additional negative effect on bone homeostasis needs to be confirmed with further translational studies. Such studies merit special consideration, as many patients are currently prescribed recombinant EPO.

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Fig. 1. Three-dimensional representative micro-CT images of femurs from wt, Vav-Cre / JAK2^{V617F/+} mice indicated as JAK2^{V617F/+}, and tg6 mice. *Top images* depict cortical bone at mid-diaphysis and *bottom images* trabecular bone at distal metaphysis

Fig. 2.

a Bone volume ratio, **b** trabecular number, **c** trabecular separation, **d** trabecular thickness in distal metaphysis, and **e** cortical bone volume ratio, **f** cortical thickness in mid-diaphysis of the femur as determined by micro-CT analysis. Wt mice shown as *black bars*, Vav-Cre / JAK2^{V617F/+}, indicated as JAK2^{V617F/+}, shown as *light gray bars*, and tg6 shown as *dark gray bars*. All parameters are presented as mean ± SD of 10 animals per group. *P < 0.05, **P < 0.01. *P < 0.05, **P < 0.01 p values indicate statistical significance between an animal

model (Vav-Cre/JAK2^{V617F/+} or tg6) vs. wt mice. Additional statistical differences between Vav-Cre /JAK2^{V617F/+} and tg6 animals are depicted on each figure

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Fig. 3.

a Representative images (original magnification 40×; scale bar= 25 μm) of H&E staining of longitudinal sections through the tibia of wt, Vav-Cre /JAK2^{V617F/+} indicated as JAK2^{V617F/+}, and tg6 mice. Histologic analysis quantification of **b** percentage of bone volume per tissue volume (BV/TV), **c** trabecular number (TbN), **d** number of osteoblasts per tissue area (NOb/TAR), **e** percentage of osteoid volume per bone volume (OV/ BV), **f** osteoid thickness (OTh), **g** percentage of bone surface occupied by osteoblasts (ObS/BS). All parameters are presented as mean±SD of 10 animals per group. **h** Double fluorescent

labeling with calcein injected 7 and 2 days prior to sacrifice depicted as bone formation rate per tissue volume (BFR/TV). All parameters are presented as mean±SD. Wt shown as *black bars*, Vav-Cre /JAK2^{V617F/+}, indicated as JAK2^{V617F/+}, shown as *light gray bars*, and tg6 shown as *dark gray bars*. * $P<0.05$, ** $P<0.01$, * $P<0.05$, ** $P<0.01$ p values indicate statistical significance between an animal model (Vav-Cre/JAK2^{V617F/+} or tg6) vs. wt mice. Additional statistical differences between Vav-Cre /JAK2^{V617F/+} and tg6 animals are depicted on each figure

Fig. 4.

Histologic analysis quantification of **a** number of osteoclasts per bone perimeter (NOc/BPm), **b** percentage of bone surface occupied by osteoclasts (OcS/BS). All parameters are presented as mean±SD of at least seven animals per group. **c-d** PMMA-embedded longitudinal femur sections were stained for TRAP. **c** Number of osteoclasts per bone perimeter (NOc/BPm) and **d** percentage of bone surface occupied by osteoclasts (OcS/BS) were determined. All measurements were done using cellSens standard software. All parameters are presented as mean±SD of at least nine animals per group. Wt shown as *black bars*, Vav-Cre / JAK2^{V617F/+}, indicated as JAK2^{V617F/+}, shown as *light gray bars*, and tg6 shown as *dark gray bars*. No statistical differences were detected among groups. **e** Representative images (original magnification 10×; scale bar= 100 μm) of longitudinal femur sections stained for TRAP of wt, Vav-Cre / JAK2^{V617F/+} indicated as JAK2^{V617F/+}, and tg6 mice