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ORIGINAL ARTICLE

Activin-A is elevated in patients with thalassemia major and double heterozygous sickle cell/beta-thalassemia and correlates with markers of hemolysis and bone mineral density

Ersi Voskaridou[1] · Ioannis Ntanasis-Stathopoulos[2] · Dimitrios Christoulas[3] · Maria Dimopoulou[1] · Veroniki Komninaka[1] · Konstantina Repa[1] · Athanasios Papatheodorou[4] · Evangelos Terpos[2]

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Abstract

Despite the advances in the management of hemoglobinopathies, further insight into disease pathophysiology is necessary to improve our therapeutic approach. Activin-A has emerged as a regulator of erythropoiesis and bone turnover in malignant disorders; however, clinical data in hemoglobinopathies are currently scarce. Thus, we aimed to investigate the role of activin-A among hemoglobinopathy patients and evaluate the rationale of its targeting. Circulating levels of activin-A were measured in patients ($n = 227$) with beta-thalassemia major (TM) ($n = 58$), beta-thalassemia intermedia (TI) ($n = 43$), double heterozygous sickle cell/beta-thalassemia (HbS/beta-thal) ($n = 109$), or homozygous sickle cell disease ($n = 17$), and we explored possible correlations with clinical and laboratory data. Seventeen age- and gender-matched, healthy individuals served as controls. Bone marrow density (BMD) was determined using dual-energy X-ray absorptiometry. TM and HbS/beta-thal patients had elevated activin-A compared to controls ($p = 0.041$ and $p = 0.038$, respectively). In TM patients, high circulating activin-A showed strong correlations with hemolysis markers, namely reticulocyte count ($p = 0.011$) and high lactate dehydrogenase (LDH; $p = 0.024$). Similarly, in HbS/beta-thal patients, activin-A showed positive correlations with indirect bilirubin ($p < 0.001$), ferritin ($p = 0.005$), and LDH ($p = 0.044$). High activin-A correlated with low Z-score of both lumbar spine BMD in TI patients ($p < 0.01$) and femoral neck BMD in TM patients ($p < 0.01$). Serum activin-A is elevated in patients with TM and HbS/beta-thal and correlates with markers of hemolysis and low BMD. These data support a role of activin-A in the biology of these disorders and provide further rationale for the broader clinical development of activin-A inhibitors in this setting.

Keywords Activin · Hemoglobinopathy · Thalassemia · Sickle cell disease · Bone · Hemolysis

Introduction

Hemoglobinopathies are widely acknowledged as the most frequently encountered inherited diseases around the globe with as many as 400,000 affected newborns per year [1]. Advances in transfusion regimens and iron overload monitoring have significantly contributed to alleviate the disease burden in terms of survival and quality of life [2]. Although allogeneic stem cell transplantation and gene therapy in a limited number of selected patients may provide a hope for cure [3], further insight into disease pathophysiology is deemed necessary in order to find novel and more effective therapeutic approaches for the majority of patients.

The transforming growth factor beta (TGF-β) superfamily has been recognized as a potent regulator of hematopoiesis and is directly implicated in the disease phenotype of hemoglobinopathies [4, 5]. Activin-A is a member of this large superfamily and has a multifaceted function by regulating erythropoiesis, hormonal homeostasis, musculoskeletal tissue repair, bone metabolism, neuronal interactions, and carcinogenesis [6]. Several studies have documented the

✉ Evangelos Terpos
eterpos@med.uoa.gr; eterpos@hotmail.com

[1] Thalassaemia and Sickle Cell Disease Reference Centre, Laikon General Hospital, Athens, Greece

[2] Department of Clinical Therapeutics, School of Medicine, Alexandra General Hospital, National and Kapodistrian University of Athens, 80 Vas. Sofias Avenue, 11528 Athens, Greece

[3] Department of Haematology, 251 General AirForce Hospital, Athens, Greece

[4] Department of Medical Research, 251 General AirForce Hospital, Athens, Greece

erythropoietic effects of activin-A in transformed cell lines or other in vitro models [6]; however, there is a relative paucity of functional data regarding hematopoietic roles of activin-A in vivo. Especially among patients with hemoglobinopathies, the data are scarce. Regarding bone remodeling, activin A is produced by osteoblasts, osteoclasts, and bone marrow cells, and there is agreement that activin-A promotes osteoclast development in vitro [7]; nevertheless, the effect of activin-A on osteoblast development in vitro varies significantly depending on experimental conditions [8–10].

Activin-A exerts its function through binding to the activin type IIA (ActRIIA) and type IIB (ActRIIB) receptors [6]. Interestingly, ActRIIA and ActRIIB ligand traps have shown significant activity in restoring dyserythropoiesis in several disease contexts ranging from thalassemia to myelodysplastic syndromes and multiple myeloma [11–14]. Despite their undoubtful role in anemia-related disorders including hemoglobinopathies, their exact mechanism of action remains currently rather elusive [6].

Taking into consideration all the above, the aim of the present study was to evaluate activin-A in different types of hemoglobinopathies in an attempt to characterize its role in ineffective erythropoiesis and bone disease. Such a pathophysiological insight would significantly add to the limited available literature data and enhance the rationale of the use of activin-A antagonists in this cohort of patients.

Patients and methods

This was a prospective study for the evaluation of circulating activin-A levels in the serum of patients diagnosed with hemoglobinopathies and their correlation with disease features and biochemical parameters.

Study participants

Eligible patients were considered those with a definite diagnosis of hemoglobinopathy, including beta-thalassemia major (TM), thalassemia intermedia (TI), double heterozygous sickle cell/beta-thalassemia (HbS/β-thal), and homozygous sickle cell disease (HbS/HbS). Activin-A was also measured in the serum of 17 age- and gender-matched healthy individuals, without any known comorbidities or drug administration. All patients and controls provided their written informed consent for blood sampling and for recording their pertinent medical data.

The study was conducted according to the principles defined by the 18th World Medical Association Assembly (Declaration of Helsinki, 1964) and all its future amendments. The study protocol was designed and executed according to the national guidelines and regulations along with the Good Clinical Practice Guidelines as defined by the International Conference of Harmonization. The study was approved by the institutional ethics committee.

Study endpoints

The primary endpoint of the present study was the assessment of circulating serum activin-A levels in patients diagnosed with hemoglobinopathies and the comparisons with healthy controls of similar gender and age as well as among disease subtypes, namely TM, TI, HbS/β-thal, and HbS/HbS.

Secondary endpoints pertained to the assessment of the association of activin-A levels with (a) ferritin, (b) hemolysis indices (reticulocyte percentage, lactate dehydrogenase [LDH], indirect bilirubin), and (c) bone disease, as reflected by bone mineral density (BMD), in each disease subgroup.

Measurement of activin-A and biochemical indices

Activin-A levels and biochemical parameters were measured in all patients and controls. Regarding activin-A evaluation, serum was separated within 4 h following vein puncture and it was stored at − 80 °C until the day of measurement. An ELISA methodology (Quantikine, R&D Systems, Minneapolis, MN, USA) was implemented. The assay recognizes natural and recombinant activin-A. Importantly, no significant cross-reactivity or interference with other activin classes or members of the TGF superfamily has been observed (Quantikine® ELISA, Catalog Number DAC00B, Package insert 2017, R&D Systems, Minneapolis, MN, USA). Biochemical indices, including ferritin, reticulocyte percentage, LDH, and indirect bilirubin, were assessed at the day of vein puncture.

Evaluation of bone disease

The disease burden on bone integrity was evaluated by BMD of lumbar spine (L1–L4) and femoral neck (FN). BMD was determined using dual-energy X-ray absorptiometry (DEXA; LUNAR, PRODIGY Version 8.60.006/SYSTEM GE medical system LUNAR USA 726, Madison, WI, USA) at the time of activin-A measurement. The presence and severity of osteoporosis were defined according to the World Health Organization (WHO) definition based on DEXA data [15].

Data recording and statistical analysis

Clinical patient data were collected from the medical files. Source data verification was implemented and, thus, data accuracy was assured. A two-sided p value of less than 0.05 was considered statistically significant and confidence intervals (CI) referred to 95% boundaries in all performed statistical tests. Statistical analysis was performed using SPSS 21.0 (IBM Corp., Armonk, NY).

Results

Patient characteristics

Two hundred twenty-seven adult patients with hemoglobinopathies were included in the present study. The majority of them were females ($n = 173$, 76.2%). The age range of the study population was 19–69 years. Regarding the distribution of hemoglobinopathy subtypes, 58 patients were diagnosed with TM and 43 with TI, whereas 109 had HbS/β-thal and 17 had HbS/HbS. Detailed patient characteristics are provided in Table 1, whereas Table 2 shows the comparisons between patient hemoglobinopathy subgroups regarding ferritin levels, markers of hemolysis, and bone disease.

Activin-A levels according to disease subgroup

Activin-A levels varied across patient subgroups and controls (Fig. 1). Patients with TM (mean ± SD, 481 ± 213 pg/ml) and HbS/beta-thal (459 ± 181 pg/ml) had elevated circulating activin-A compared to controls (361 ± 87 pg/ml; $p = 0.041$ and $p = 0.038$, respectively, Fig. 1). However, circulating activin-A levels did not differ significantly between TI patients (427 ± 509 pg/ml) and controls ($p = 0.202$) or between SCD patients (422 ± 132 pg/ml) and controls ($p = 0.811$, Fig. 1). Furthermore, TM patients had higher activin-A levels compared to patients with TI ($p = 0.002$), whereas TI patients showed lower activin-A levels compared to HbS/β-thal ($p < 0.001$). No other significant differences in the levels of activin-A between patient subgroups according to hemoglobinopathy subtype were observed (Table 2). Among TM patients, activin-A levels were also inversely correlated with hemoglobin levels ($p = 0.04$), whereas the associations among the remaining patient subgroups were non-significant.

Correlation of activin-A levels with ferritin and hemolysis indices

In patients with TM, high circulating activin-A showed strong correlations with markers of hemolysis, such as elevated reticulocyte counts ($r = 0.406$, $p = 0.011$, Fig. 2a) and high LDH ($r = 0.397$, $p = 0.024$, Fig. 2b). Regarding HbS/beta-thal patients, activin-A showed positive correlations with ferritin ($r = 0.270$, $p = 0.005$, Fig. 3a), indirect bilirubin ($r = 0.399$, $p < 0.001$, Fig. 3b), and LDH ($r = 0.194$, $p = 0.044$, Fig. 3c).

Association of activin-A levels with bone disease

According to BMD *T*-score values, osteoporosis was present in 45% of patients with TM, in 40% of those with TI, in 33% of SCD patients, and in 25% of patients with HbS/β-thal (Fig. 4). Interestingly, high activin-A levels correlated with low *Z*-score of L1–L4 BMD in TI patients ($r = 0.615$, $p < 0.01$, Fig. 5a) and low *Z*-score of FN BMD in TM patients ($r = 0.456$, $p < 0.01$, Fig. 5b).

Discussion

The present prospective study provides a further insight into the role of activin-A in the pathogenesis of hemoglobinopathies and potentiates the rationale for targeting its signaling pathway in terms of therapeutics. To the best of our knowledge, this is the first clinical report in the field encompassing data from 227 patients.

We found elevated circulating serum activin-A levels in patients diagnosed with TM and HbS/β-thal compared to controls, respectively. Interestingly, both TM and HbS/β-thal showed increased activin-A levels compared to TI patients, respectively. This finding may be associated with the well-established differences in the pathophysiological basis between TM or HbS/β-thal and TI. It can be postulated that TI patients have overall better compensatory mechanisms due to the presence of the normal allele and, thus, activin-A levels are not substantially deregulated [16]. On the contrary, SCD patients did not show significant differences in measured activin-A levels as compared to controls. Apart from the relatively small number of included SCD patients in our study, it should be noted that SCD has a completely distinct biological background from various types of thalassemia. More specifically, neither ineffective erythropoiesis nor bone disease is among the cardinal features of the disease; therefore, activin-A levels were expectedly not increased [17].

Furthermore, activin-A levels were positively correlated with hemolysis markers in both TM and HbS/β-thal patient groups. Such an association has been also described previously in a clinical setting among pregnant women in pre-eclamptic state and those with HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome [18]; however, this is the first referral regarding patients with hemoglobinopathies. Hemolysis is among the principal manifestations of both entities but with different underlying mechanisms. In TM, the relative excess of the alpha subunit of the hemoglobin tetramer results in the precipitation of the alpha chains inside the erythroid precursor cells, formation of reactive oxygen species, and the subsequent distortion of the cellular membrane, which in turn causes cell lysis [19]. In SCD, the landmark mutation of the β-globin gene leads to the formation of hemoglobin S that is characterized by a high degree of oxidative stress, and of sickle cells that present with defective deformability. Sickle cells are getting entrapped in the spleen and they are subsequently destroyed (extravascular hemolysis), whereas intravascular hemolysis takes place also and contributes to the disease burden [19]. Taking into consideration the lack of pertinent literature data, it may be postulated either that activin-A

Table 1 Overview of patient characteristics; age is expressed as median (range), sex distribution as absolute number (*n*), and the other parameters as mean values ± SD

Parameters	TM (*n* = 58)	TI (*n* = 43)	HbS/β-thal (*n* = 109)	HbS/HbS (*n* = 17)
Age (years)	42 (29–66)	49 (27–67)	46 (19–69)	45 (32–68)
Sex (M/F)	26/32	11/32	43/66	4/13
Transfusion-dependent (*n*)	58	0	0	0
Ferritin (ng/ml)	1349 ± 1348	591 ± 1316	606 ± 785	693 ± 866
Activin-A (pg/ml)	481 ± 213	427 ± 509	459 ± 181	422 ± 132
Hemolysis indices				
Reticulocytes (%)	6.6 ± 7.7	5.2 ± 4.8	5.4 ± 3.4	7.2 ± 5.5
LDH	226 ± 95	352 ± 185	348 ± 123	435 ± 191
Indirect bilirubin	0.77 ± 1.22	0.69 ± 0.65	0.57 ± 0.61	0.71 ± 0.42
BMD (*T*-score)				
L1-L4	− 1.98 ± 1.06	− 1.81 ± 0.45	0.48 ± 2.17	0.89 ± 3.37
Femoral neck	− 1.7 ± 1.03	− 1.51 ± 0.74	0.41 ± 4.16	0.89 ± 3.85

TM beta-thalassemia major; *TI* thalassemia intermedia; *HbS/β-thal* double heterozygous sickle cell/beta-thalassemia; *HbS/HbS* homozygous sickle cell disease; *M* males; *F* females; *LDH* lactate dehydrogenase; *BMD* bone marrow density

plays a role in the disruption of cellular membrane in the thalassemic microenvironment or that there are other confounding factors that may mediate the observed association. Therefore, the potential underlying pathophysiological mechanisms have to be elucidated in future studies.

Although there have been accumulating data on the role of activin-A on hemo- and erythropoiesis, its exact role remains rather controversial. Activin-A was initially referred as erythroid differentiation factor (EDF) implicated in the maturation and differentiation of red blood cells [20]. Functionally, activin-A is pleiotropic and affects different cell types within the hematopoietic system, mainly the B lymphocyte and the erythroid lineages, particularly the multipotential and erythroid progenitor cells [21]. During embryonic development, activin-A has been shown to be important for the differentiation of mesodermal precursors to hemangioblast fate [22]. In adult life, activin-A is constitutively expressed by stromal cells in the hematopoietic microenvironment [23], whereas its expression is low to undetectable in hematopoietic stem cells (HSCs), but it is subsequently induced during hematopoietic differentiation [24, 25].

Evidence from in vitro and in vivo studies support activin-A as an erythropoiesis stimulatory factor [26, 27]. Activin-A has been shown to enhance erythroid differentiation of primitive human hematopoietic cells in vitro [23, 28, 29]. Activin A is implicated at earlier stages of erythropoiesis by enhancing the formation of BFU-E [27, 30, 31]. Activin-A also induces the differentiation of mouse Friend erythroleukemia cells (MEL) at low concentrations (1–10 ng/ml); however, it suppresses their growth in soft agar [32]. This effect has been also demonstrated in human K562 cells along with a simultaneous increase in hemoglobin synthesis [33], as well as in the transcription of globin genes [34]. The effect of activin-A on erythropoiesis has been also shown through its administration

Table 2 *p* values of comparisons between patient hemoglobinopathy subgroups with regard to activin-A, LDH, indirect bilirubin, ferritin levels, reticulocyte percentage, L1–L4, and femoral neck BMD *T*-score. Italicized values denote statistical significance

Parameters	TM vs TI	TM vs HbS/β-thal	TM vs HbS/HbS	TI vs HbS/β-thal	TI vs HbS/HbS	HbS/β-thal vs HbS/HbS
Activin-A	*0.002*	0.487	0.369	*< 0.001*	0.090	0.600
Ferritin	*< 0.001*	*< 0.001*	*0.020*	0.389	0.135	0.484
Hemolysis indices						
Reticulocytes	0.726	0.155	0.112	0.120	0.057	0.234
LDH	*< 0.001*	*< 0.001*	*< 0.001*	0.389	0.065	0.068
Indirect bilirubin	0.549	*0.026*	0.505	*0.002*	0.805	*0.023*
BMD (*T*-score)						
L1–L4	0.552	*< 0.001*	*0.015*	*< 0.001*	*0.029*	0.852
Femoral neck	0.226	*0.034*	0.246	0.185	0.350	0.749

TM beta-thalassemia major; *TI* thalassemia intermedia; *HbS/β-thal* double heterozygous sickle cell/beta-thalassemia; *HbS/HbS* homozygous sickle cell disease; *LDH* lactate dehydrogenase; *BMD* bone marrow density

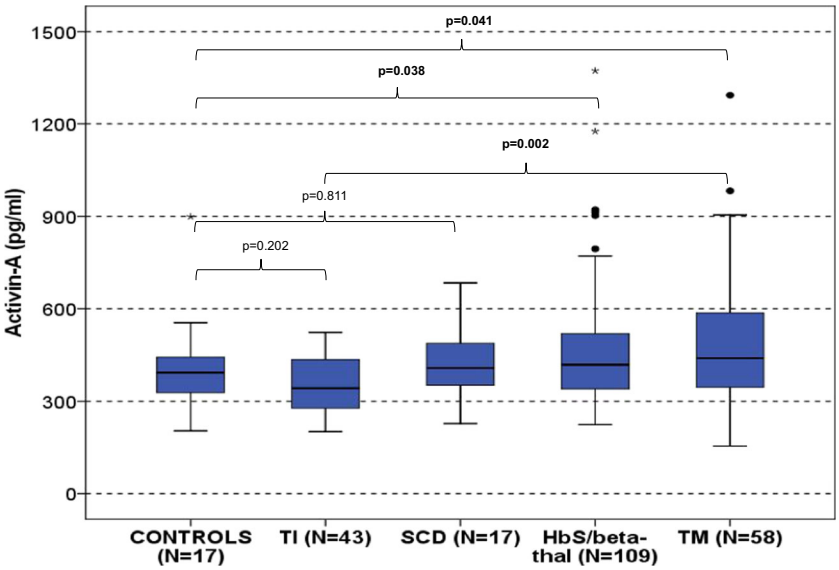

Fig. 1 Forest plot of activin-A levels (pg/ml) among patients with hemoglobinopathies (TM, TI, HbS/beta-thal, SCD) and controls. Bold values denote statistical significance

to rodents. Such in vivo studies have shown an increase in erythroid precursors [27], circulating red blood cells [35], and reticulocyte release [36] upon treatment with activin-A, whereas follistatin, an activin-A inhibitor, causes a reduction in progenitor numbers [26].

However, there is evidence that activin-A exerts an erythropoiesis inhibitory role as well. In vitro addition of activin-A to an erythroid colony assay system resulted in the suspension of erythroid colony-forming unit (CFU-E) colony formation and this effect was dose-dependent [31]. In vitro co-culture of activin-A and murine hematopoietic stem cells inhibited the proliferation of the latter [25]. Additionally, as aforementioned, activin-A suppressed the growth of MEL in soft agar [32]. In our study, activin-A levels were inversely correlated with hemoglobin levels among patients with TM, supporting the inhibitory effect on erythropoiesis. However, literature data on the correlation of activin-A levels with markers of ineffective/expanded erythropoiesis, such as soluble transferrin receptor 1, are currently lacking and should be investigated in future studies.

Taking all the above into consideration, it seems that activin-A may have a differential effect on hemopoiesis; this can be explained by the interrelated effects of all the members of the TGF-β superfamily. TGF-β superfamily members including TGF-β, activins, and bone morphogenetic proteins (BMPs) are known to be present in the bone marrow microenvironment and have been implicated in stem cell differentiation and hematopoiesis through their convergent regulation of SMAD intracellular cascade [37]. Both the intracellular crosstalk among molecular pathways along with the intercellular interactions between hemopoietic cells and the microenvironment point towards a multivariate, context-dependent

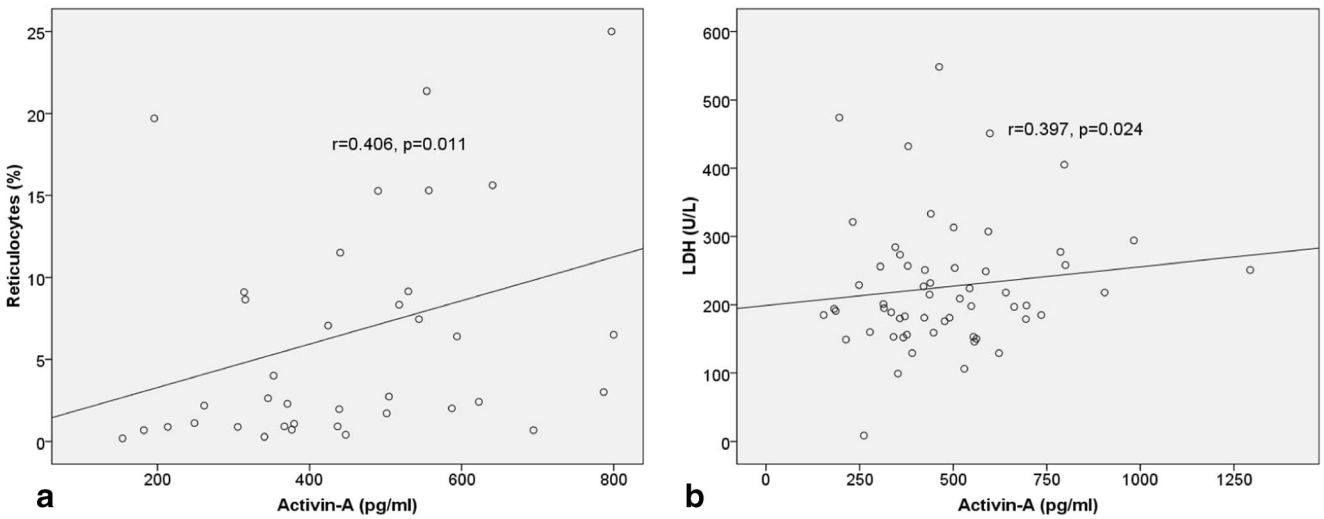

Fig. 2 Circulating activin-A levels were strongly correlated with **a** reticulocyte count ($r = 0.406$, $p = 0.011$) and **b** high LDH ($r = 0.397$, $p = 0.024$) in patients with TM

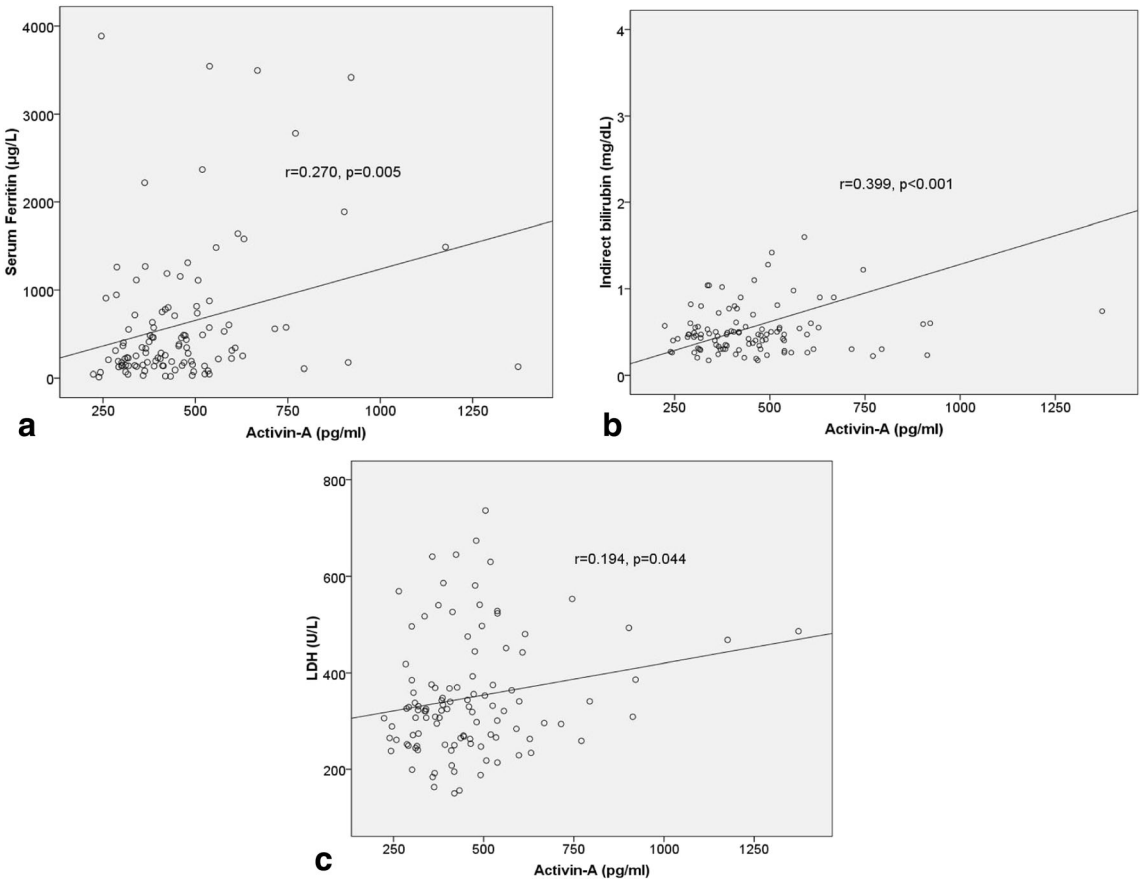

Fig. 3 Circulating activin-A levels were strongly correlated with **a** ferritin ($r = 0.270$, $p = 0.005$), **b** indirect bilirubin ($r = 0.399$, $p < 0.001$), and **c** LDH ($r = 0.194$, $p = 0.044$) levels in patients with HbS/beta-thal

effect of activin-A that has to be further elucidated in future studies with an integrative approach [37].

Regarding bone disease, elevated activin-A levels were significantly associated with low *Z*-score of FN BMD and L1–L4 BMD among TM and TI patients, respectively. Similarly, this inverse association between activin-A levels and BMD has been also described in postmenopausal women [38]. Furthermore, increased activin-A levels have been associated with increased bone resorption and extensive bone disease among patients with multiple myeloma [39, 40], whereas elevated values have been also identified among patients with bone metastases due to breast or prostate carcinomas [41]. In thalassemia, bone disease is multifactorial including both disease- and therapy-related aspects; nevertheless, the main regulators of bone metabolism remain the same, namely osteoclasts and osteoblasts [42]. Activin-A along with other members of the TGF-β superfamily are actively implicated in the regulation of bone turnover [10]. It has been clearly demonstrated that activin-A induces osteoclast differentiation by promoting the receptor activator of nuclear factor kappa B (NF-κB) ligand (RANKL)-induced osteoclastogenesis through the C-FOS and SMAD signaling pathways [7]. Regarding the role of activin-A in osteoblast formation, the interactions are more complex. Although there are data showing a favorable effect by prolonging osteoblast survival [8], contradictory evidence suggests that activin-A suppresses both osteoblast differentiation and bone mineralization [9, 40, 43]. Moreover, activin-A has been shown to increase sclerostin expression, which is a strong inhibitor of the WNT signaling pathway and, subsequently, of osteoblast differentiation [44]. Interestingly, sclerostin has been detected in

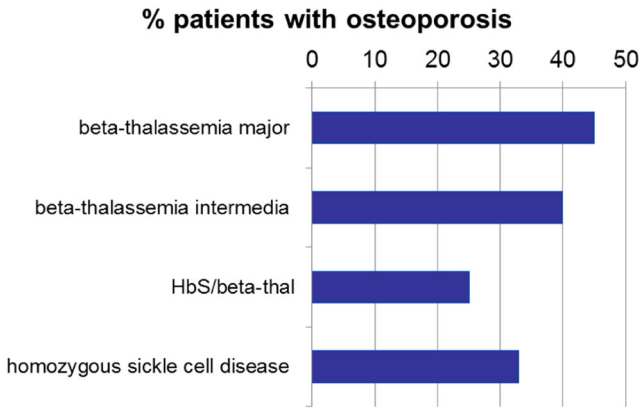

Fig. 4 Distribution of osteoporosis among patients with hemoglobinopathies

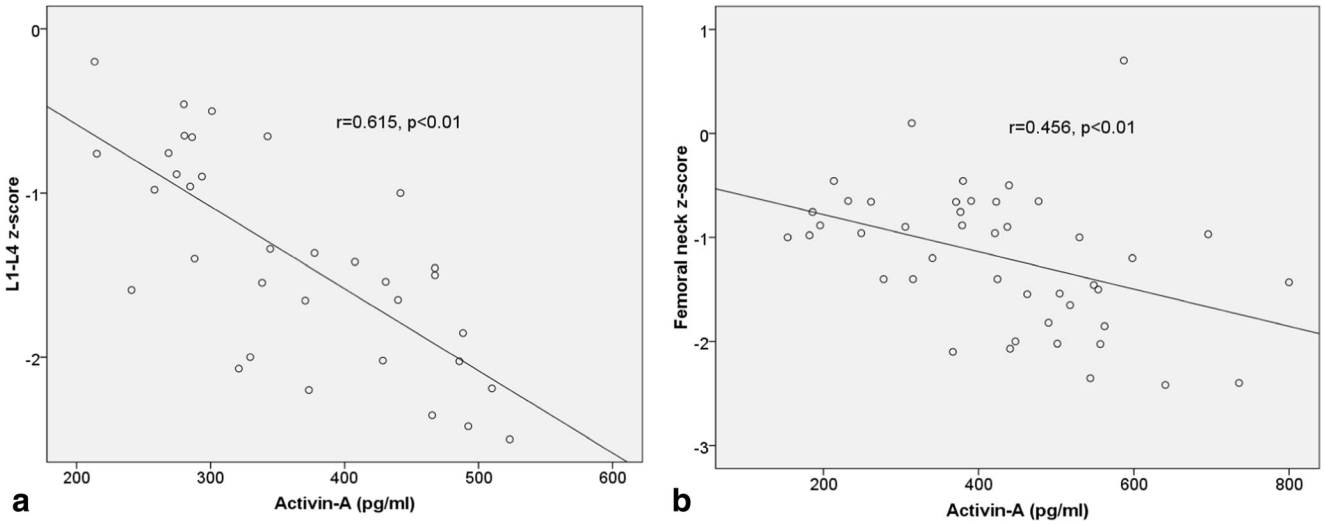

Fig. 5 Activin-A levels were correlated with **a** *Z*-score of L1–L4 BMD in TI patients ($r = 0.615$, $p < 0.01$) and **b** *Z*-score of FN-BMD in TM patients ($r = 0.456$, $p < 0.01$)

high levels among patients with thalassemia-induced osteoporosis and low BMD values [45]. In this context, the findings of the present study should be considered even more valuable, since they can be integrated into the previous knowledge and, thus, they provide a novel insight into the pathogenesis of bone disease in thalassemia patients.

As aforementioned, activin-A, along with other members of the TGF-β pathway, exerts its downstream effects through binding to the ActRIIA and ActRIIB receptors [6]. The most promising therapeutic approach in targeting these pathways has been the development of ligand traps. The two main representatives of this class of drugs are sotatercept (ACE-011) and luspatercept (ACE-536) [11]. The encouraging preclinical findings have provided the rationale for the conduction of clinical studies in thalassemia patients that have defective erythropoiesis (Table 3).

Sotatercept is a fusion protein consisted of the Fc portion of the human immunoglobulin IgG1 combined with a part of the extracellular domain of the human ActRIIA. It acts as a sequestering agent for the ligands of the ActRIIA [11]. Initial attempts were performed in the context of treating bone-related disorders; however, clinical studies in postmenopausal women unexpectedly revealed a favorable effect on erythropoiesis indices [46]. Similar results have been also revealed in patients with multiple myeloma treated with sotatercept that showed improvement in both bone formation markers and hemoglobin levels [12]. Preclinical studies with sotatercept have demonstrated an induction of late-stage erythropoiesis, the subsequent release of mature erythrocytes into circulation, and restoration of anemia. This effect is at least partially mediated by the inhibition of growth differentiation factor 11 (GDF-11) which is an ActRIIA ligand. Suppressing the function of GDF-11 leads to decreased intracellular oxidative stress and α-globin precipitation [47]. Another preclinical study confirmed that the inhibition of activin-A and GDF-11 by sotatercept promoted the sustainable differentiation and survival of erythroid progenitor cells [48]. Interestingly, there are also data underlining the role of microenvironment by providing evidence that the effects of sotatercept are largely dependent on bone marrow accessory cells and mediating factors [48–50]. Sotatercept has also shown to regulate hepcidin levels of hepatocytes and exert a favorable role on

Table 3 Clinical trials evaluating the role of sotatercept and luspatercept in hemoglobinopathies according to Clinicaltrials.gov.

Clinicaltrials.gov registration number	Title	Status
NCT01571635	Study to Determine the Safety and Tolerability of Sotatercept (ACE-011) in Adults With Beta (β) - Thalassemia.	Active, not recruiting
NCT03342404	A Study to Determine the Efficacy and Safety of Luspatercept in Adults With Non Transfusion Dependent Beta (β)-Thalassemia (BEYOND)	Recruiting
NCT02604433	An Efficacy and Safety Study of Luspatercept (ACE-536) Versus Placebo in Adults Who Require Regular Red Blood Cell Transfusions Due to Beta (β) Thalassemia (BELIEVE)	Active, not recruiting
NCT01749540	Study to Evaluate the Effects of ACE-536 in Patients With Beta-thalassemia	Completed
NCT02268409	ACE-536 (NCT01749540) Extension Study - Beta Thalassemia	Active, not recruiting

hemoglobin levels among hepcidin transgenic mice; however, further research is needed in order to clarify this aspect and the probable effect on thalassemia subjects [51, 52]. Furthermore, the interim results of an ongoing phase 2a clinical trial including adult patients with β-thalassemia have been very encouraging (NCT01571635). Sotatercept increased hemoglobin levels and decreased the transfusion burden, providing sustainable results in a dose-dependent manner, whereas it was well tolerated and demonstrated a favorable safety profile [53].

Luspatercept is a modified ActRIIB ligand trap with similar structure to sotatercept and inactivates members of the TGF-β superfamily that bind to ActRIIB [11, 54]. In preclinical studies, luspatercept has shown to alleviate defective erythropoiesis and decrease hemolysis indices by inhibiting SMAD signaling and restoring GATA-1 function [55, 56]. Apart from erythropoiesis-inducing effects, luspatercept has also demonstrated a restoration of thalassemia-associated complications including bone disease, splenomegaly, and iron overload [4, 55]. In the clinical setting, promising results of phase 2 studies of luspatercept in patients with β-thalassemia have indicated an overall decrease of disease and transfusion burden, including anemia, iron overload, and leg ulcers, along with an improvement in quality-of-life and physical activity indices (NCT02268409, NCT02604433) [57–59].

In conclusion, we showed that increased activin-A levels were detected in patients with TM and HbS/β-thal and they were associated with increased hemolysis indices. Furthermore, elevated activin-A was inversely correlated with BMD among thalassemia patients. Thus, we reinforce the rationale for targeting activin-A and its downstream effectors in order to counteract both defective erythropoiesis and thalassemia-induced bone disease. Our data strongly support designing relative clinical trials with activin-A inhibitors alone or in combination with other agents in hemoglobinopathies, while circulating activin-A levels may be used as a surrogate marker of efficacy in order to elucidate its exact role in disease pathophysiology.

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Author contributions EV and ET were the principal investigators and designed the study. ET and INS took primary responsibility for the paper and wrote it. All authors collected and analyzed the data. DC performed the statistical analyses. All authors contributed to the critical revision and approved the final version to be published.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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