Osteoporos Int
DOI 10.1007/s00198-016-3883-1

ORIGINAL ARTICLE

Birmingham epidermolysis severity score and vitamin D status are associated with low BMD in children with epidermolysis bullosa

G. Rodari[1] · S. Guez[2] · F. Manzoni[2] · K. K. Chalouhi[3] · E. Profka[1] · S. Bergamaschi[1] · S. Salera[2] · G. Tadini[2] · F. M. Ulivieri[4] · A. Spada[1] · C. Giavoli[1] · S. Esposito[2]

Received: 2 August 2016 / Accepted: 12 December 2016

Abstract
Summary Bone status impairment represents a complication of generalized forms of epidermolysis bullosa (EB); however, the prevalence and the main determinants of this event in localized forms remain poorly defined. Birmingham epidermolysis bullosa severity (BEBS) score and 25-hydroxyvitamin D levels are strongly associated with low bone mass, suggesting that vitamin D may play a potential beneficial role in bone health. Further longitudinal studies are needed in order to confirm this hypothesis.
Introduction Bone status impairment represents a complication of generalized forms of EB; thus, we aimed to estimate the prevalence of low bone mass, to examine mineralization differences in various EB subtypes and to identify the most important determinants of bone impairment in children with either generalized or localized EB.
Methods An observational study of 20 children (11 males; mean age ± standard deviation, 11.7 ± 3.9 years) with EB was performed. Clinical history, physical examination, laboratory studies, X-ray of the left hand and wrist for bone age, and dual energy X-ray absorptiometry scans of the lumbar spine were obtained. Areal bone mineral density (aBMD Z-scores) and bone mineral apparent density were related to the BEBS score.
Results Areal BMD Z-score (mean −1.82 ± 2.33, range, −7.6-1.7) was reduced (<−2 SD) in 8 patients (40%), whereas aBMD Z-score adjusted for bone age was low in 7 patients (35%). BEBS score and 25-hydroxyvitamin D serum levels were the most important elements associated with aBMD ($P = 0.0001$ and $P = 0.016$, respectively). A significant correlation between the aBMD Z-score and area of skin damage, insulin-like growth factor-1, C-reactive protein, and sodium serum levels was also found.
Conclusions Low aBMD can be considered a systemic complication of EB, primarily associated with BEBS score and 25-hydroxyvitamin D levels. Therefore, longitudinal evaluation of bone status is ongoing in these patients to define whether vitamin D supplementation would prevent, or at least reduce, bone status impairment.

Keywords BEBS score · Bone mineral density · Epidermolysis bullosa · Osteoporosis · Pediatrics · Vitamin D

This work was supported by Ricerca Corrente Funds of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan.

✉ C. Giavoli
claudia.giavoli@gmail.com

[1] Endocrinology and Metabolic Diseases Unit, Department of Clinical Sciences and Community Health, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[2] Pediatric Highly Intensive Care Unit, Department of Pathophysiology and Transplantation, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[3] Department of Radiology, IRCCS Policlinico San Donato, University of Milan, San Donato Milanese, Milan, Italy

[4] Bone Metabolic Unit, Division of Nuclear Medicine, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Introduction

The term epidermolysis bullosa (EB) refers to a group of extremely rare mechanobullous genodermatoses characterized by varying degrees of skin and mucous membrane fragility, which readily blisters in response to minor mechanical trauma [1, 2]. Pathogenetic mutations of EB involve genes encoding

Published online: 23 December 2016

for structural proteins within the epidermis, skin basement membrane zone (dermoepidermal junction), or uppermost dermis. Referring to the location of the target proteins and to the level of blisters, four major subtypes of EB have been identified: EB simplex (EBS), junctional EB (JEB), dystrophic EB (DEB), and Kindler syndrome (KS), which are characterized by mixed levels of blistering [3, 4].

Although EB is often viewed as a skin disease, the most severe forms, such as recessive dystrophic EB (RDEB) or JEB, are actually multisystemic disorders with extracutaneous complications, such as esophageal strictures, cardiomyopathy, renal insufficiency, anemia, and growth failure [5, 6]. Pathological reduction of bone mass might be included among systemic manifestations and can result from failure to gain adequate peak bone mass (PBM) from premature bone loss or from a combination of both processes. Suboptimal acquisition of adequate PBM, the amount of bone tissue reached at the end of skeletal maturation, is an immediate and future concern in terms of fracture risk [7].

As with many chronic diseases, children with EB are frequently malnourished (either for the high metabolic demands or for the reduced nutritional intake, as in the presence of esophageal strictures or gastrointestinal malabsorption) and can show a severe failure to thrive and delayed pubertal development [5, 6]. Moreover, 25-hydroxyvitamin D (25(OH) D) serum levels are commonly low due to the presence of skin blisters and bandages interfering with cutaneous vitamin D production [5, 6, 8]. Moreover, restricted physical activity and chronic inflammatory state along with elevated cytokine concentrations might interfere with peak bone mass achievement [8, 9].

Only four studies thus far have examined bone involvement in children with EB; all have had a relatively small sample of patients due to the rarity of the disease and have only focused on the severe and generalized forms. All reports, though not homogeneous in study design and evaluated parameters, concluded that EB patients often have low bone mass for age, but no clear determinant for this risk has been identified [10–13].

In the present study, we aimed to estimate the prevalence of low bone mass, to examine mineralization differences in various EB subtypes, and to identify the most important clinical or laboratory determinants of bone status impairment in children with either generalized or localized EB.

Methods

Subjects

We performed this observational study at the Center for Epidermolysis in Northern Italy at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan. The study was approved by the Ethics Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Parental written informed consent was obtained from parents before enrolment; moreover, children ≥8 years were required to give their written assent before entering the study.

A total of 20 EB children (11 males; mean age ± standard deviation [SD], 11.7 ± 3.9 years) were enrolled. Inclusion criterion was age above 6 years. Ongoing steroid treatment and vitamin D supplementation were exclusion criteria. Out of 20 subjects, 10 were affected with RDEB, 4 by DEB, 3 by EBS, 2 by KS, and 1 by JEB. Clinical phenotype, skin biopsy, and genetic mutation analysis supported the diagnosis in accordance with the Third International Consensus Meeting on Diagnosis and Classification of EB [14].

Examinations

Each patient completed a thorough history, physical examination, and laboratory studies. Height was obtained with a stadiometer and compared with Tanner Height Charts, and weight and body mass index (BMI) Z-score were determined using the Centers for Disease Control and Prevention 2000 growth charts [15]. All patients underwent a left wrist and hand radiography, which was interpreted according to the standards of Greulich and Pyle [16] to obtain bone age. Bone age was considered delayed if it was more than 12 months younger than chronologic age. Pubertal development was assessed and compared with Tanner stages [17, 18]. EB severity was evaluated following the Birmingham epidermolysis bullosa severity (BEBS) score, which includes not only the area of skin damage but also nail and mucosal involvement, scarring of hands, skin cancer, chronic wounds present for at least 6 months, alopecia, and nutritional compromise. The term "area of skin damage" included blisters, erosions, scabs, healing skin, erythema, and atrophic scarring and excluded skin changes not resulting directly from damage, such as mottled pigmentation in EBS and poikiloderma in KS [19]. Mobility was scored using a categorical scale (0 = immobile; 1 = occasional wheelchair use; 2 = ambulatory but not active; 3 = fully active). Each patient underwent a validated nutritional questionnaire [20] to assess daily calcium intake according to the International Institute of Medicine standards [21].

Laboratory studies included calcium, phosphorus, magnesium, sodium, alkaline phosphatase (ALP) and isoenzymes, C-reactive protein (CRP), 25(OH) D, 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), parathyroid hormone (PTH), thyroid stimulating hormone (TSH), free T4, insulin-like growth factor-1 (IGF-1), luteinizing hormone (LH), follicle stimulating hormone (FSH), 17 β-estradiol (in females), testosterone (in males), urinary calcium, and creatinine ratio from fasting sample. Serum

calcium was corrected for albumin concentration (4.4 g/dL). In particular, 25(OH) D levels were measured by commercial radioimmunoassay (Diasorin, Saluggia, Italy), and a chemiluminescent immunoenzymatic assay was used to assess serum PTH concentrations (LIAISON N-TACT PTH Assay, Diasorin, Stillwater, MN, USA) and IGF-1 levels (Immulite 2000 IGF1, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). All the other parameters were measured following standard procedures by the central laboratory of our hospital.

Dual energy X-ray absorptiometry (DXA) scans of the lumbar spine (Hologic Discovery bone densitometers-*software version* 3.3 APEX, Discovery A, Hologic Inc., Marlborough, MA, USA) were obtained from the study patients. Areal BMD (aBMD) Z-scores were calculated based on chronological age adjusted for bone age when bone age was at least 12 months <chronological age and for height (to correct possible underestimation in the presence of short stature), as previously described [22]. However, these methods are not free from interpretation defects, with a particular concern for the use of aBMD Z-score height because short-for-age children are compared with children of similar height but earlier stage of sexual maturation.

From DXA scans, bone mineral apparent density (BMAD, g/cm^3) was also determined, thus obtaining a potential correction method in an attempt to approximate the true volumetric density in children with short stature, based on the assumption that the vertebral body is a cylinder [22, 23]. A low aBMD Z-score for chronological age, bone age, and height was defined as aBMD Z-score of less than or equal to −2 [24].

Statistical analysis

Statistical analysis was performed using SPSS version 21.0 statistical package (SPSS IBM, New York, USA). Descriptive statistics (mean ± SD and range) were used to characterize the patient population and bone health results. The comparison between aBMD Z-score for chronological age, bone age, or height and laboratory or auxological variables was performed using Pearson's bivariate correlation analysis. The one-way ANOVA test and Bonferroni post hoc analysis were used to compare linear variables and nominal multiple variables (i.e., BMD vs. mobility score). Student's *t* test was performed for means comparisons (i.e., mean aBMD Z-score in male and female patients or mean chronologic age vs. mean bone age). A stepwise regression method was used to assess risk factors associated with low aBMD for chronological age. The predictor variables tested included the BEBS score, area of skin involvement, corrected calcium, sodium, 25(OH) D, IGF-1 SD, CRP, and BMI Z-score. Stepwise removal was performed with exclusion criterion $P > 0.10$. Statistical significance was defined as a two-sided $P < 0.05$.

Results

Characteristics of the study population

The clinical, biochemical, and densitometric characteristics of the study population are reported in Table 1. Overall, 12 of 20 patients (60%) were prepubertal, and among them, 2 children showed an actual condition of delayed puberty (Tanner breast stage I above the age of 13 in female and testicular volume less than 4 mL at 14 years old in male). Of the remaining 8 children (7 of them in Tanner stage II, 1 in Tanner stage III), 2 had a delayed progression of pubertal development (Tanner stage II at the age of 19.2 years and Tanner stage III at the age of 18.9 years). Pathological short stature (height SD <−2 SD) was found in 5 of 20 patients (25%). Skeletal maturation was commonly delayed (in 7 of 17 patients, 41%). With the exception of three RDEB patients in whom bone age could not be assessed in the presence of pseudosyndactyly and hand scars, the mean bone age (BA) was significantly different from the mean chronological age (9.52 ± 3.22 years vs. 11.7 ± 3.89 years, respectively, $P = 0.0018$). Regarding physical activity, no patient was completely immobile, but 5 (25%) occasionally used a wheelchair, 7 (35%) were ambulatory but not active, and 8 patients (40%) were capable of playing sports. None of the patients had a history of pathological fractures.

Daily calcium intake questionnaire showed a dietary insufficiency in 14 (70%) of 20 EB children (655 ± 410 mg, 80% population recommended intake).

Mean IGF-1 was low for age, at less than −2 SD in 8 of 20 patients (40%). After correction for albumin (4.4 g/dL), calcium was normal in all patients. A total of 19 patients (95%) had serum 25(OH) D concentrations less than 30 ng/mL (75 nmol/L); in particular, in 6 patients (30%), 25(OH) D was between 20 and 30 ng/mL (50–75 nmol/L), in 13 patients (65%), it was less than 20 ng/mL (50 nmol/L), and in 9 patients (45%), it was below 12 ng/mL (30 nmol/L). Serum PTH and ALP were in the normal range in all patients. Regarding densitometric data, the aBMD Z-score was pathologically (aBMD Z-score < −2) reduced in 8 of 20 patients (40%) and in 7 of 20 (35%) when corrected for BA (aBMD Z-score BA).

The Pearson correlation test highlighted a significant negative correlation between height Z-score and BEBS score ($r^2 = 0.63$, $P < 0.0001$, Fig. 1a). Indeed, 14 of 19 children (74%) showed a short stature compared with the genetic target (presence of a negative difference between height Z-score and target Z-score obtained with a midparental height formula) that was inversely correlated with the BEBS score ($r^2 = 0.43$, $P = 0.003$, Fig. 1b).

Table 1 Clinical, laboratory, and densitometric data in the study population

Clinical data	Mean ± SD	Range
Chronological age (CA), year	11.7 ± 3.89	7–19
Bone age (BA), year	9.52 ± 3.22	6–18
Height, cm	137 ± 15	115–162
Height Z-score	−1.23 ± 2.15	−5.7 − +1.9
Weight, kg	30 ± 10	17–49
Weight Z-score	−2.62 ± 4.16	−13.5 − +2.2
BMI[a], kg/m^2	15.65 ± 3.61	10.8–23
BMI[a] Z-score	−2.03 ± 2.98	−9.2 − +2.3
BEBS[b]	25.2 ± 21.44	3–63
Area of skin damage	10.9 ± 9.37	1–28
Laboratory	**Mean ± SD**	**Nv**
Calcium, mg/dL	9.25 ± 0.75	8.4–10.4
Calcium correct, mg/dL	9.58 ± 0.37	8.4–10.4
IGF-1[c], ng/mL	160 ± 115	
IGF-1[c] SD	−1.42 ± 1.54	
25 (OH) D[d], ng/mL	15.06 ± 9.53	>20
1,25(OH)$_2$D$_3$[e], pg/mL	55.5 ± 20.3	16–81
CRP[f], ng/mL	2.14 ± 2.82	<0.5
Sodium, mEq/L	138 ± 3.23	135–145
PTH[g], pg/mL	32.4 ± 10.6	15–65
Fosforum, mg/dL	4.35 ± 0.56	3–5
Magnesium, mg/dL	2.08 ± 0.12	1.73–2.26
ALP[h], U/L	170 ± 58	<300
U-calcium/U-creatinin	0.11 ± 0.12	<0.2
TSH[i], uU/mL	3.13 ± 1.21	0.28–4.3
LH[l] , mU/mL	0.8 ± 1.4	0.1–5.1
FSH[m], mU/mL	1.8 ± 1.4	0.7–6.1
Testosterone, ng/mL	0.8 ± 1.5	0.02–4.7
17β-estradiol, pg/mL	8.3 ± 5.1	5–17
Densitometric data	**Mean ± SD**	**Range**
Area, cm^2	39.11 ± 7.92	28.59–53.27
BMC[n], g	21.99 ± 8.71	10–41.7
BMD[o], g/cm^2	0.548 ± 0.120	0.318–0.783
aBMD[p] Z-score	−1.82 ± 2.33	−7.6–1.7
aBMD[p] Z-score bone age	−1.45 ± 2.26	−7.6–1.7
aBMD[p] Z-score height	−1.11 ± 1.79	−4.7–3.9
BMAD[q], g/cm^3	0.261 ± 0.056	0.162–0.362

[a] Body mass index
[b] Birmingham epidermolysis bullosa severity score
[c] Insulin-like growth factor-1
[d] 25-hydroxyvitamin D
[e] 1,25-dihydroxyvitamin D3
[f] C-reactive protein
[g] Parathyroid hormone
[h] Alkaline phosphatase
[i] Thyroid-stimulating hormone
[l] Luteinizing hormone
[m] Follicle-stimulating hormone
[n] Bone mineral content
[o] Bone mineral density
[p] Areal bone mineral density
[q] Bone mineral apparent density

Correlations with densitometric data

Referring to the different EB subtypes, our results show that more severe forms had more bone involvement (Fig. 2).

Pearson's bivariate correlation test was performed to assess which factors might interfere with mineralization. By this analysis, the aBMD Z-score was negatively correlated with the BEBS score ($r^2 = 0.74$, $P < 0.0001$, Fig. 3a) and area of skin damage ($r^2 = 0.66$, $P < 0.0001$, Fig. 3b), but positively correlated with 25(OH) D levels ($r^2 = 0.57$, $P = 0.0001$, Fig. 3c), IGF-1 SD ($r^2 = 0.64$, $P < 0.0001$), and the BMI Z-score ($r^2 = 0.56$, $P = 0.0001$). Moreover, CRP ($r^2 = 0.48$, $P = 0.001$) and sodium values ($r^2 = 0.45$, $P = 0.001$) correlated with the aBMD Z-score, negatively and positively, respectively. Serum calcium concentrations did not show a significant relationship with the aBMD Z-score.

According to backward stepwise regression, the BEBS score ($B = -0.07 \pm 0.14$, $P = 0.0001$) and 25(OH) D serum levels ($B = 0.09 \pm 0.032$, $P = 0.016$) were the most important elements associated with aBMD. Disease severity was inversely associated with mineralization, with a 0.7 decrease in the aBMD Z-score for every ten-point increase in the BEBS score. Moreover, serum 25(OH) D concentrations correlated significantly with the BEBS score ($r^2 = 0.39$, $P = 0.003$, VIF = 1, Fig. 3c) and extent of blistering ($r^2 = 0.49$, $P = 0.0006$, Fig. 3d).

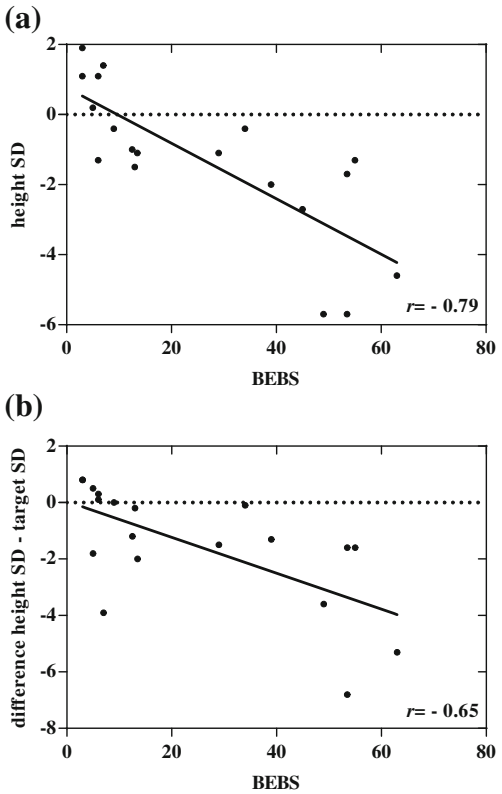

Fig. 1 Auxological data and BEBS score **a** correlation with height SD and **b** difference (height SD−target SD)

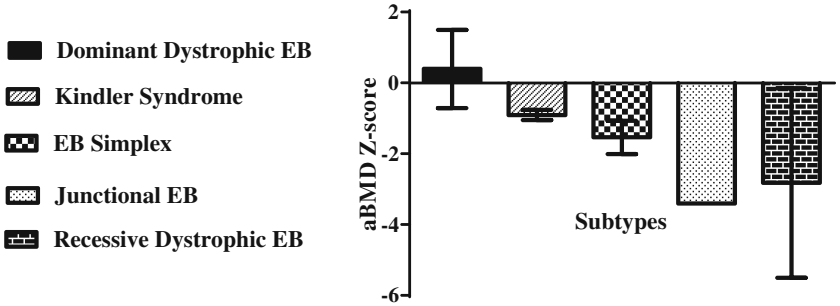

Fig. 2 Mineralization in different epidermolysis bullosa (EB) subtypes

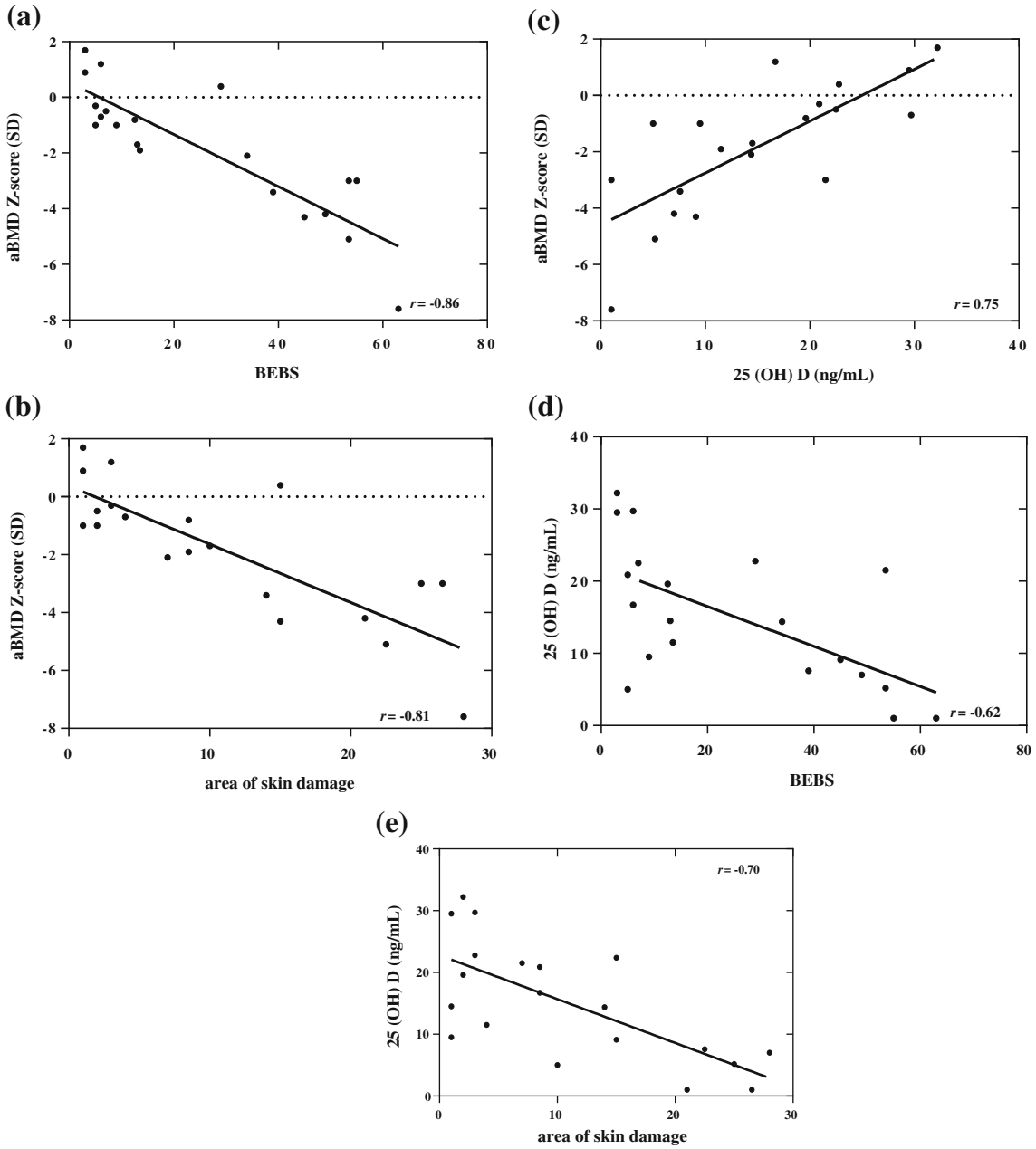

Fig. 3 Main determinants of areal bone mineral density (aBMD). **a** Correlation with BEBS score. **b** Area of skin damage. c 25(OH) D (**c**). **d** Correlation between 25(OH) D and BEBS score. **e** Area of skin damage

Immobility was another important factor negatively influencing bone status (Fig. 4). Sorting patients into four groups, mobility rate was positively correlated with 25(OH) D levels ($P = 0.007$, Fig. 5). Moreover, a positive significant association between weight-bearing activity and aBMD Z-score ($P = 0.001$) was found by one-way ANOVA test. According to Bonferroni post hoc analysis, the most important differences were observed between the extremes, i.e., between groups 1 and 3 ($P = 0.001$). The aBMD Z-scores BA were slightly lower than the aBMD Z-scores, although all the previous correlations were reconfirmed. Moreover, BMAD significantly correlated with the aBMD Z-score ($r^2 = 0.76$, $P < 0.0001$).

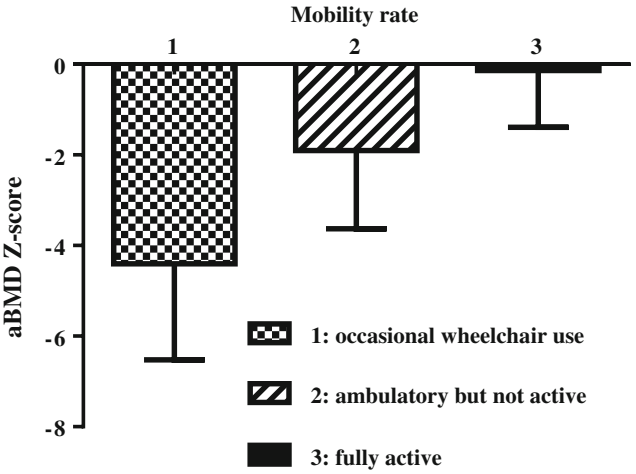

Fig. 4 Association between physical activity and areal bone mineral density (aBMD): correlation with mobility rate

Discussion

This study showed that in children with EB, low bone mass is frequent and strongly related to the BEBS score and low vitamin D levels. To the best of our knowledge, this is the first report on this topic evaluating children with either generalized or localized forms of EB classified according to the BEBS score, a valid and reproducible tool that, due to the lack of a genotype-phenotype correlation, more strictly reflects disease severity [19].

As previously mentioned, the four studies published to date on this topic concordantly identified reduced bone mass as a complication of generalized forms of EB. However, a comparison between the present results and each of those already published was not always possible, either due to laboratory data or densitometric parameters that were not shown for all patients or referred to different normal values in previous studies [10–13]. In this regard, Reyes et al. in a cross-sectional, observational study of 7 children with generalized forms of EB found low BMD in 3 patients (43%) and low serum 25(OH) D levels in four [10]. In a retrospective study of 39 EB patients, Fewtrell et al. found that children with RDEB and JEB had lower BMD than age-matched control subjects or EBS patients, although only a few patients underwent laboratory examination in this study [11]. Bruckner et al. reported a low aBMD in 24 children with severe, generalized forms of EB [12]. In addition, they showed that 25(OH) D serum levels and IGF-1 concentrations were frequently low but, contrary to our results, they showed no correlation with the aBMD Z-score [12]. Finally, Fu et al. followed a cohort of 17 patients with generalized EB for 12 months and in most subjects observed an increase in absolute aBMD, although the patients failed to gain as much as expected for their ages, resulting in a decrease in the aBMD Z-score [13]. Consistent with previous reports [10–12], many patients included in the present study had a low aBMD Z-score (40%) and aBMD Z-score BA (35%), suggesting that despite being traditionally viewed as a skin disease, EB actually represents a multisystemic disorder that can adversely influence skeletal health.

This study allowed us to identify clinical and laboratory factors that might interfere with adequate bone mineral accrual, such as 25(OH) D concentration, nutritional state (indirectly assessed by BMI and IGF-1 Z-score), mobility rate, and CRP. An inflammatory state mirrored by CRP elevation might be involved in bone health and BMD reduction, as found in many other chronic diseases such as inflammatory bowel disease [25]. Moreover, different inflammatory processes blended with autoimmune events have been demonstrated in inherited EB, revealing an overlapping with EB acquisita. Autoantibodies, which are the primary cause in EB acquisita, can be secondary produced as a result of genetically determined skin damage in inherited EB, worsening the disease clinical manifestation and the inflammatory state [26]. As far as sodium concentrations, sodium values were frequently at the lower limit of the normal range or frankly reduced in our patients and were positively correlated with the aBMD Z-score. Although no study has assessed this correlation yet, hyponatremia might affect skeletal health because sodium is capable of binding hydroxyapatite crystal, and bone tissue represents the principal site of sodium storage. Low serum

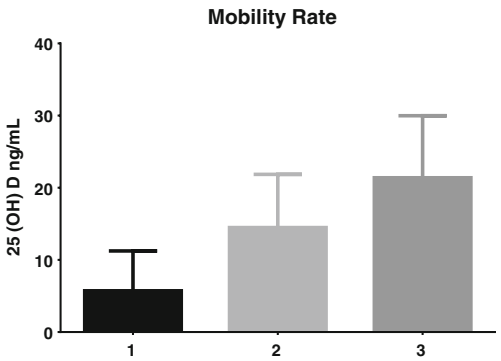

Fig. 5 Association between 25(OH) D levels and mobility rate

25(OH) D was the most important laboratory parameter associated with bone metabolism. Although vitamin D deficiency is commonly defined by 25(OH) D serum concentrations below 20 ng/mL (50 nmol/L) [8, 21, 27–31], to date, no consensus exists on 25(OH) D sufficiency levels. The Institute of Medicine and the Pediatric Endocrine Society consider sufficient 25(OH) D concentrations above 20 ng/mL and severe vitamin D insufficiency for 25(OH) D levels below 12 ng/mL [21, 27, 28]. In contrast, the Endocrine Society, the International Osteoporosis Foundation, and studies on pediatric populations promote a desirable 25(OH) D threshold of 30 ng/mL (75 nmol/L) [29–31]. Thus, levels between 20 and 30 ng/mL might reflect a vitamin D insufficiency [8, 29–31]. In our study population, 60% of patients had 25(OH) D concentrations below 20 ng/mL, which is commonly considered a state of deficiency. Among them, 45% of patients had 25(OH) D levels below 12 ng/mL, reflecting a severe state of vitamin D insufficiency. This observation is consistent with data reported in adults affected with autoimmune bullous skin diseases, such as pemphigus vulgaris and bullous pemphigoid [32, 33] but seems to be in contrast with previous studies performed in EB children that did not report a correlation between low vitamin D levels and mineralization [11, 12]. This discrepancy might be partly explained by the inclusion in the present study of patients with either generalized or localized forms of EB and with KS, thus leading to more variability in 25(OH) D concentrations. Moreover, three of the four previously published studies [11–13] were performed in the USA, where food is routinely supplemented with vitamin D, whereas fortified food is not commonly available in Italy, and our patients were not supplemented with vitamin D. Although the daily calcium intake questionnaire showed a dietary insufficiency in 14 of 20 EB children (70%), serum-corrected calcium concentrations were normal in all the patients and were not associated with the aBMD Z-score, suggesting that in these children, calcium homeostasis could be preserved to the detriment of calcium bone storage. Consistently, PTH and ALP concentrations were normal in all patients, different from what was observed by Reyes et al. [10], but in accordance with the studies published by Fewtrell et al. [11] and Bruckner et al. [12].

ABMD might be underestimated in children with delayed BA, and for this reason, the aBMD Z-score data were adjusted for BA. After correction for BA, bone mineral density remained frequently low, and all the correlations were reconfirmed, strengthening our results. The determination of BMAD helped us to avoid a possible underestimation of the aBMD Z-score in the presence of short stature. The significant correlation found between these parameters further supports an actual alteration in mineralization.

The present study has some limitations, such as the relatively small sample of patients due to the rarity of the disease and the lack of data on vertebral fractures. In fact, considering disease severity and poor health conditions, an X-ray of the lumbar spine was not performed in asymptomatic study patients. Two children with severe back pain underwent X-ray of the dorsal and lumbar spine (aBMD Z-score CA −7.6 and −3.9, respectively), but vertebral fracture was not detected. However, on the basis of a very recent study highlighting a consistent relationship between BMD Z-scores and vertebral fractures [34], we intend to perform spinal radiographs, or a morphometric DXA evaluation, during the follow-up of patients with low BMD Z-scores to define the best therapeutic approach.

In conclusion, low bone mineral density can be considered an additional multifactorial complication of EB. BEBS score and 25(OH) D levels emerged as strongly associated with bone impairment, suggesting a potential beneficial role of vitamin D supplementation, disease control, adequate nutritional state, and improvement of mobility rate in bone mineralization in these patients. Further studies are needed in order to verify the impact of vitamin D supplementation in this population and to establish whether bone defects reflect a real increase in fracture risk.

Compliance with ethical standards We performed this observational study at the Center for Epidermolysis in Northern Italy at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan. The study was approved by the Ethics Committee of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. Parental written informed consent was obtained from parents before enrolment; moreover, children ≥8 years were required to give their written assent before entering the study.

Conflicts of interest None.

References

1. Fine JD (2010) Inherited epidermolysis bullosa: recent basic and clinical advances. Curr Opin Pediatr 22:453–458
2. Tadini G, Gualandri L, Colombi M, Paradisi M, Angelo C, Zambruno G et al (2005) The Italian registry of hereditary epidermolysis bullosa. G Ital Dermatol Venereol 140:359–372
3. Intong LR, Murrell DF (2012) Inherited epidermolysis bullosa: new diagnostic criteria and classification. Clin Dermatol 30:70–77
4. Fine JD, Bruckner-Tuderman L, Eady RA, Bauer EA, Bauer JW, Has C et al (2014) Inherited epidermolysis bullosa: updated recommendations on diagnosis and classification. J Am Acad Dermatol 70:1103–1126
5. Fine JD, Mellerio JE (2009) Extracutaneous manifestations and complications of inherited epidermolysis bullosa: part I. Epithelial associated tissues. J Am Acad Dermatol 61:367–384
6. Fine JD, Mellerio JE (2009) Extracutaneous manifestations and complications of inherited epidermolysis bullosa: part II. Other organs. J Am Acad Dermatol 61:387–402
7. Bachrach LK (2001) Acquisition of optimal bone mass in childhood and adolescence. Trends Endocrinol Metab 12:22–28
8. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357:266–281

9. Annicchiarico G, Morgese MG, Esposito S, Lopalco G, Lattarulo M, Tampoia M et al (2015) Proinflammatory cytokines and antiskin autoantibodies in patients with inherited epidermolysis bullosa. Medicine (Baltimore) 94:e1528
10. Reyes ML, Cattani A, Gajardo H, Garcia C, McGrath JA, Palisson F (2002) Bone metabolism in children with epidermolysis bullosa. J Pediatr 140:467–469
11. Fewtrell MS, Allgrove J, Gordon I, Brain C, Atherton D, Harper J et al (2006) Bone mineralization in children with epidermolysis bullosa. Br J Dermatol 154:959–962
12. Bruckner L, Bedocs LA, Keiser E, Tang JY, Doernbrack C, Arbuckle HA et al (2011) Correlates of low bone mass in children with generalized forms of epidermolysis bullosa. J Am Acad Dermatol 65:1001–1009
13. Fu T, Lingala B, Kent K, Bachrach LK, Bruckner AL (2011) Pattern of bone mineral acquisition in children with epidermolysis bullosa: a longitudinal study. Br J Dermatol 165:1081–1086
14. Fine JD, Eady RA, Bauer EA, Bauer JW, Bruckner-Tuderman L, Heagerty A et al (2008) The classification of inherited epidermolysis bullosa (EB): report of the Third International Consensus Meeting on diagnosis and classification of EB. J Am Acad Dermatol 58:931–950
15. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R et al (2000) CDC growth charts: United States. Adv Data 314:1–27
16. Greulich WW, Pyle SL (1959) Radiographic atlas of skeletal development of the hand and wrist. 2nd ed. Stanford University Press, Stanford
17. Marshall WA, Tanner JM (1970) Variation in pattern of pubertal changes in boys. Arch Dis Child 45:13–23
18. Marshall WA, Tanner JM (1969) Variation in pattern of pubertal changes in girls. Arch Dis Child 44:291–303
19. Moss C, Wong A, Davies P (2009) The Birmingham epidermolysis bullosa severity score: development and validation. Br J Dermatol 160:1057–1065
20. Montomoli M, Gonnelli S, Ghiacchi M, Mattei R, Cuda C, Rossi S et al (2002) Validation of a food frequency questionnaire for nutritional calcium intake assessment in Italian women. Eur J Clin Nutr 56:21–30
21. Institute of Medicine (IOM) (2011) Dietary reference intakes for calcium and vitamin D. The National Academies Press, Washington, pp. 260–262
22. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S et al (2010) Height adjustment in assessing dual energy X-ray absorptiometry measurements of bone mass and density in children. J Clin Endocrinol Metab 95:1265–1273
23. Bianchi ML (2007) Osteoporosis in children and adolescents. Bone 41:486–495
24. Official Pediatric Position of the ISCD. Skeletal Health Assessment In Children from Infancy to Adolescence, 2013
25. Paganelli M, Albanese C, Borrelli O, Civitelli F, Canitano N, Viola F et al (2007) Inflammation is the main determinant of low bone mineral density in pediatric inflammatory bowel disease. Inflamm Bowel Dis 13:416–423
26. Esposito S, Guez S, Manzoni F, Bosco A, Rigante D (2015) Epidermolysis bullosa and the partnership with autoimmunity: what should we assimilate? Immunol Res 61:63–69
27. Bouillon R, Van Schoor NM, Gielen E, Boonen S, Mathieu C, Vanderschueren D et al (2013) Optimal vitamin D status: a critical analysis on the basis of evidence-based medicine. J Clin Endocrinol Metab 98:1283–1304
28. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M (2008) Drug and Therapeutics Committee of the Lawson Wilkins, Pediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics 122:398–417
29. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP et al (2012) Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J Clin Endocrinol Metab 97:1153–1158
30. Vierucci F, Del Pistoia M, Fanos M, Gori M, Carlone G, Erba P et al (2013) Vitamin D status and predictors of hypovitaminosis D in Italian children and adolescents: a cross-sectional study. Eur J Pediatr 172:1607–1617
31. Marchisio P, Consonni D, Baggi E, Zampiero A, Bianchini S, Terranova L et al (2013) Vitamin D supplementation reduces the risk of acute otitis media in otitis-prone children. Pediatr Infect Dis J 32:1055–1060
32. Marzano AV, Trevisan V, Eller-Vainicher C, Cairoli E, Marchese L, Morelli V et al (2012) Evidence for vitamin D deficiency and increased prevalence of fractures in autoimmune bullous skin diseases. Br J Dermatol 167:688–691
33. Marzano AV, Trevisan V, Cairoli E, Eller-Vainicher C, Morelli V et al (2015) Vitamin D and skeletal health in autoimmune bullous skin diseases: a case control study. Orphanet J Rare Dis 10:8
34. Ma J, Siminoski K, Alos N, Halton J, Ho J, Lentle B, Matzinger M et al (2015) The choice of normative pediatric reference database changes spine bone mineral density Z-scores but not the relationship of bone mineral density with prevalent vertebral fractures. J Clin Endocrinol Metab 100:1018–1127