

Natural history of growth and anaemia in children with epidermolysis bullosa: a retrospective cohort study*

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Summary

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Conflicts of interest

None to declare.

A.R. initiated the study, retrieved the data and drafted the initial manuscript. Data collection during clinical visits of patients was established by A.S.-B., H.S. and D.K., and carried out by A.S.-B., D.K., H.S., F.S., A.R. and C.H. Genetic analysis of most patients was performed by C.H.; all authors participated in molecular analysis of skin samples by immunofluorescence mapping. Data analysis was performed by A.R. and M.H. A.R. and M.H. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. L.B.-T. reviewed the manuscript and coordinates the EB centre Freiburg. C.H. supervised the work, and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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[Correction added on 19 November 2020, after first online publication: Projekt Deal funding statement has been added.]

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Background Impaired growth and anaemia are major extracutaneous complications of epidermolysis bullosa (EB), but data on their development are lacking.

Objectives To determine the clinical course of growth and anaemia in children with EB and clarify the impact of nutritional compromise, inflammation and genetic factors.

Methods A retrospective study was conducted of 200 children, 157 with recessive dystrophic EB (RDEB) and 43 with junctional EB (JEB)-generalized intermediate, followed at the main referral centre in Germany. Growth charts were calculated using the modified LMS method and were correlated with parameters of anaemia, nutrition, inflammation and the molecular defect in a linear model.

Results In our cohort of patients with RDEB, weight impairment started at 12–18 months old; by the age of 10 years, 50% showed wasting. The predicted median weight at age 20 years was 35.2 kg for men and 40.1 kg for women. In JEB, growth resembled that of healthy children. Anaemia was present from the second year of life onwards in RDEB and JEB. Low levels of haemoglobin, iron, vitamin D, zinc and albumin, high levels of C-reactive protein, and absence of collagen VII correlated significantly with low weight in RDEB. No correlation was observed in JEB.

Conclusions The results highlight that nutritional compromise occurs early in children with RDEB and therefore may require interventions as of the first year or two of life.

What's already known about this topic?

- Children with epidermolysis bullosa (EB) suffer from failure to thrive and anaemia as major extracutaneous complications.
- The course of growth and the development of anaemia in EB are poorly characterized.

What does this study add?

- A molecularly well characterized cohort of 200 children with EB was followed with regard to anthropometrics, anaemia and inflammation.
- We demonstrate early onset of growth failure and anaemia, most pronounced in the subset of recessive dystrophic EB.
- Awareness of early growth delay and nutritional deficiencies will improve EB care in daily practice.

Epidermolysis bullosa (EB) is a group of genodermatoses characterized by skin fragility and blistering. Among the four types of EB,¹ recessive dystrophic EB (RDEB) and junctional EB (JEB) are associated with chronic wounds and secondary systemic involvement. Extracutaneous features such as failure to thrive and anaemia have been acknowledged,^{2–5} but the clinical course of growth has only been reported anecdotally.^{6,7} A multifactorial pathogenesis of growth impairment in EB is assumed, including increased energy demand, decreased intake due to oesophageal stenosis, tooth decay and pain, and impaired nutrient absorption.² Tube-feeding via gastrostomy for improving nutrition in RDEB has been suggested⁸ and performed with different results in small cohorts.^{6,8,9} Anaemia in EB is regarded as a result of blood loss through wounds, chronic inflammation and iron deficiency.¹⁰ A therapeutic algorithm for treating anaemia in EB has recently been suggested¹¹ and guidelines are currently being established, but evidence and details of the exact course of anaemia and associated parameters are rare.

This study depicts the natural history of growth and anaemia in children and young adults with RDEB and JEB using follow-up data of a large EB cohort from the main referral centre in Germany. We calculated growth charts and used laboratory parameters of anaemia and nutrients for correlation. Inflammation as an indicator of disease activity and the underlying molecular defect were additionally considered. The precise knowledge of the natural history of EB will aid in developing better strategies to improve growth and address chronic anaemia in EB.

Patients and methods

Dataset

All patients with RDEB-generalized severe, RDEB-generalized intermediate and JEB-generalized intermediate, aged 0–25 years presenting between February 2003 and June 2018, were included in this retrospective study. Children with JEB-generalized severe were excluded as the course has been described before.^{12,13} Bodyweight and height measurements, ethnicity and laboratory results, and the milestone clinical events:¹⁴ oesophageal stenosis (indicated by medical history and/or imaging), oesophageal dilatation, gastrostomy insertion and death were retrieved from patient records. Laboratory parameters were grouped into nutrition (total protein, albumin, vitamin D, zinc and selenium), anaemia (haemoglobin, reticulocytes, ferritin, transferrin, saturation of transferrin and iron level), and inflammation [leucocytes, C-reactive protein (CRP), IgA, IgG, IgM] and thyroid-stimulating hormone (TSH). Laboratory analyses were rarely performed within the first year of life. Nutrient intakes and extent of wounds were not systematically recorded.

The molecular diagnosis of EB was established by immunofluorescence staining¹⁵ and/or mutation analysis.^{16,17} We used the relative amount of affected protein, as determined by immunofluorescence staining, for statistical analyses – collagen VII in RDEB, and collagen XVII or laminin 332 in

JEB – classified as absent, strongly reduced, reduced, or comparable to normal. Classification into RDEB severe and intermediate was performed for each case in internal conferences based on clinical and, whenever available, molecular criteria. In the first years of life, anticipation of the severity is not always possible, because pathognomonic features are not yet apparent. Also, genotype–phenotype correlations are not always predictable, especially in cases with novel mutations.¹⁸ Therefore, the statistical analyses in this study regarded, as a whole, the group of children with RDEB.

This study was approved by the ethics committee Freiburg (vote no. 78/17) and registered with the German Clinical Trials Register (DRKS00013002).

Calculation of disease-specific growth charts

Percentiles for bodyweight, height and body mass index (BMI, kg m⁻²) were calculated separately for boys and girls with RDEB and JEB using the modified LMS (lambda–mu–sigma) method of Cole and Green¹⁹ and employing GAMLSS (generalized additive models for location, scale and shape)²⁰ in R.²¹ An underlying normal distribution with varying spread dependent on age was assumed. Missing height values were imputed based on the patients' weight using linear mixed-effects models (10% of height data). To account for bias caused by serial measurements, random effects were incorporated.²² Measurements up to the age of 25 years were included to optimize the percentile model in the adult range. We calculated 95% confidence intervals for the centile estimates based on 500 bootstrap samples. An underlying normal distribution with varying spread dependent on age was generally assumed except for weight where we employed the Box–Cox t Distribution (RDEB) and the Box–Cox power exponential distribution (JEB) based on visual inspection of the model fit with worm plots.²³ The multiethnic World Health Organization (WHO) growth reference dataset (www.who.int/childgrowth/en/ and www.who.int/growthref/en/) was used for comparison.

Modelling of the association of weight with laboratory and molecular parameters

Trend lines for laboratory parameters were estimated using GAMLSS. Errors were assumed to be normally distributed. Lines for the fifth, 50th (median) and 95th quantile were plotted. Weight measurements conducted within 180 days before or after laboratory analysis were assigned to the date of laboratory investigation. Weight Z-scores, extracted from the models used for percentile estimation of bodyweight, were plotted against laboratory parameters for each patient and time point. The association of laboratory and molecular parameters with weight was investigated using GAMLSS models, one for each parameter and for each EB subtype. Missing laboratory values were imputed using multiple imputation and a predictive mean metric.²⁴ Significance of the association of laboratory parameters with weight was judged from t-tests. Reported P-values represent averages from 100 imputations. P-

values were adjusted using Holm's method and the alpha level was set to 0.025 for each EB subtype for a global alpha level of 0.05.

Results

Patient characteristics

The cohort included 200 individuals: 157 with RDEB (81 severe, 76 intermediate) and 43 with JEB (Table 1). Results of immunofluorescence staining were available in 155 (78%), and of mutation analyses in 159 cases (80%). All RDEB cases were caused by mutations in the collagen VII gene COL7A1; cases of JEB were caused by mutations in collagen XVII, laminin and integrin genes: COL17A1 ($n = 23$), LAMB3 ($n = 10$), LAMA3 ($n = 7$), LAMC2 ($n = 1$) and ITGB4 ($n = 1$) (results not available = 1). Ethnic background was German in 75, West Asian in 44 (28 Turkish, five Syrian, seven Iraqi, two Iranian, two Afghan), Russian in 38, European-other in 24, Central/Southeast Asian in seven and African in four children (results not available = 9).

Course of growth in epidermolysis bullosa

After normal weight development within the first year, weight gain decelerated in children with RDEB around the age of 12–18 months compared with that of healthy children (Fig. 1). After the age of 8 years, half of the children with RDEB showed wasting [weight < third percentile (P3) of WHO] (Fig. 1a). For patients with RDEB, weight at 20 years was predicted as 35.2 kg for men and 40.0 kg for women (P50). Undercutting of height percentiles was noted from 7 years of

age onwards in boys and 6 years in girls. Half of children with RDEB showed stunting (height < P3 of WHO) after the age of 10 years. Our model predicted final body height for RDEB (P50) with 160.1 cm (men) and 155.7 cm (women), both below P3 of WHO (Fig. 1). Those children with weight and height within the range of healthy children mostly had RDEB-intermediate (Fig. 1a). BMI in RDEB plateaued in the range of underweight for both sexes (< 18.5 kg m⁻²) (Fig. 1a), with P50 at 13.8 kg m⁻² (men) and 15.7 kg m⁻² (women) at age 20 years.

Boys with JEB showed no alterations to normal growth patterns regarding weight and height development, while girls with JEB showed weights below or in the lower percentile ranks of healthy girls (Fig. 1b). BMI for individuals with JEB was within the range of WHO percentiles in 85% of cases.

Among children with RDEB, oesophageal stenosis as a major determinant of food uptake was reported in 100 (63.7%) and oesophageal dilatation in 55 (35%) (Table 1). Twenty-one children with RDEB had gastrostomy tubes inserted at a median age of 8.4 years (range 1.3–16.0, mean 10.2 ± 9.2 years). Gastrostomies were used for tube feeding by some patients and only for medications by others. Between gastrostomy insertion and last available measurement, four of 21 increased, four of 21 maintained and five of 21 decreased their weight percentile (no comparison possible in eight of 15). Those children who received their gastrostomies beyond the age of 8.5 years decreased in weight percentiles (details available on request to the corresponding author). There were no significant differences in weight Z-scores between those children with and without oesophageal stenosis, dilatation and gastrostomies (Fig. 2 a–c).

Table 1 Characteristics of patients included in this study

EB subtype	RDEB-generalized severe	RDEB-generalized intermediate	JEB-generalized intermediate
Patient numbers			
Total	81	76	43
Male, n (%)	38 (46.9)	44 (57.9)	20 (46.5)
Female, n (%)	43 (53.1)	32 (42.1)	23 (53.5)
Age			
Median (range), years	10.4 (0–25.0)	5.6 (0–23.8)	8.9 (0–24.9)
Mean ± SD, years	10.7 ± 0.8	7.5 ± 0.7	10 ± 1.1
EB-related milestone clinical events			
Gastrostomies, n (%)	16 (19.7)	6 (7.9)	0
Oesophageal stenosis, n (%)	58 (71.6)	42 (55.3)	0
Oesophageal dilatation n (%)	22 (27.2)	23 (30.3)	0
Lethal outcome, n (%)	5 (6.2) ^a	1 (0.1) ^b	4 (9.3) ^c
Anthropometric measurements			
Total	282	235	190
Mean per patient	3.5	3.1	4.4
Laboratory measurements			
Total	197	136	116
Mean per patient	3.7	2.2	3

EB, epidermolysis bullosa; RDEB, recessive dystrophic EB; JEB, junctional EB. ^aDeath causes: general weakness ($n = 3$), squamous cell carcinoma ($n = 1$), liver failure and suspected ileus ($n = 1$), unknown ($n = 1$). ^bDeath causes: septicemia ($n = 1$). ^cDeath causes: multiorgan failure ($n = 2$), asphyxia ($n = 1$), unknown ($n = 1$).

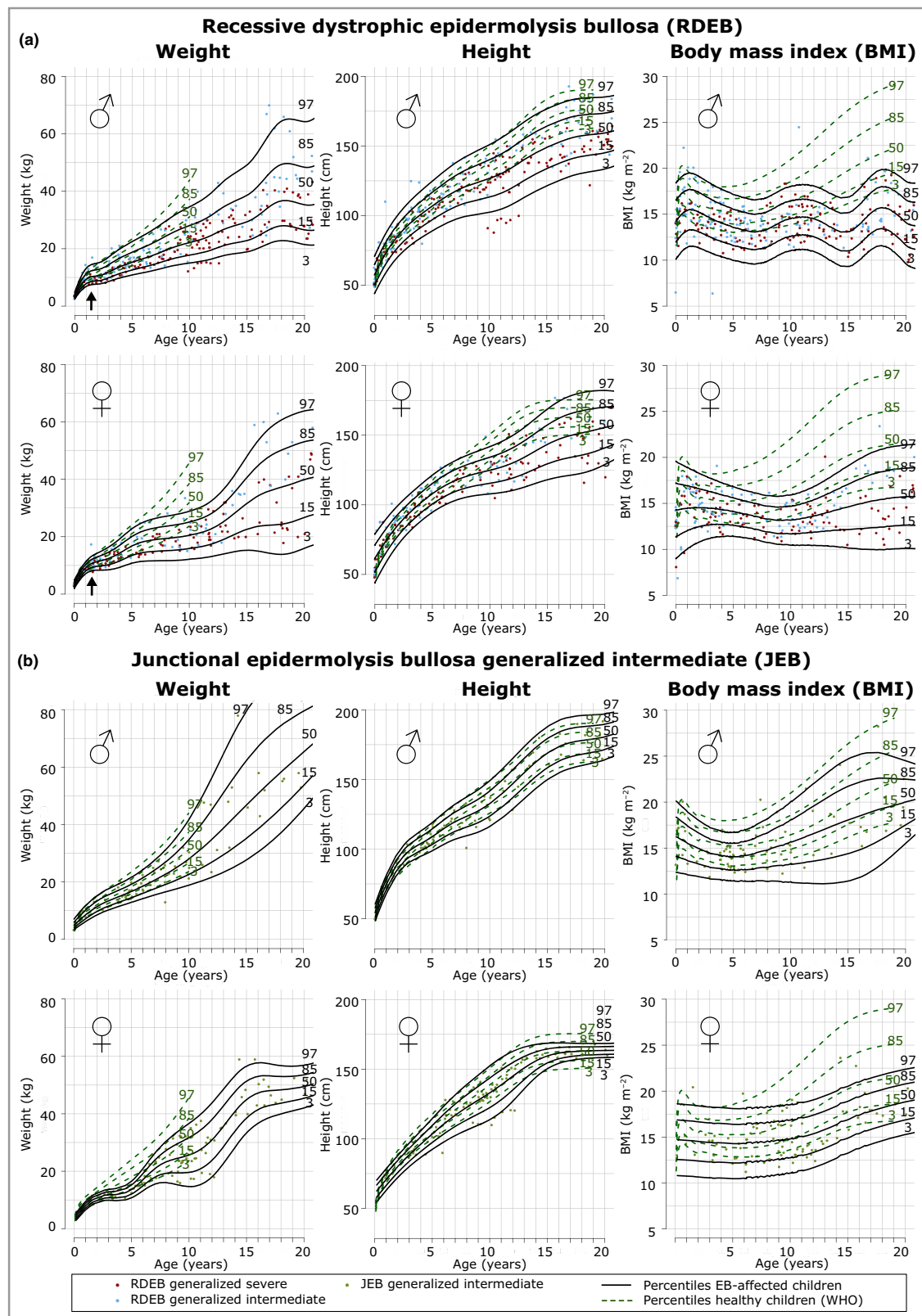


Fig 1. Growth charts of children with RDEB-generalized (a) and JEB-generalized intermediate (b), followed at the EB centre Freiburg. Weight-for-age, height-for-age and BMI-for-age charts for boys (upper panel) and girls (lower panel) with EB compared with healthy children [World Health Organization (WHO) data, green dashed lines, third, 15th, 50th, 85th and 97th percentiles; WHO weight data is only supplied up to the age of 10 years]. The onset of aberrant weight development in children with RDEB is indicated by black arrows. Individual measurements are shown by dots; the disease-specific third, 15th, 50th, 85th and 97th percentiles are indicated by black lines. [Colour figure can be viewed at wileyonlinelibrary.com]

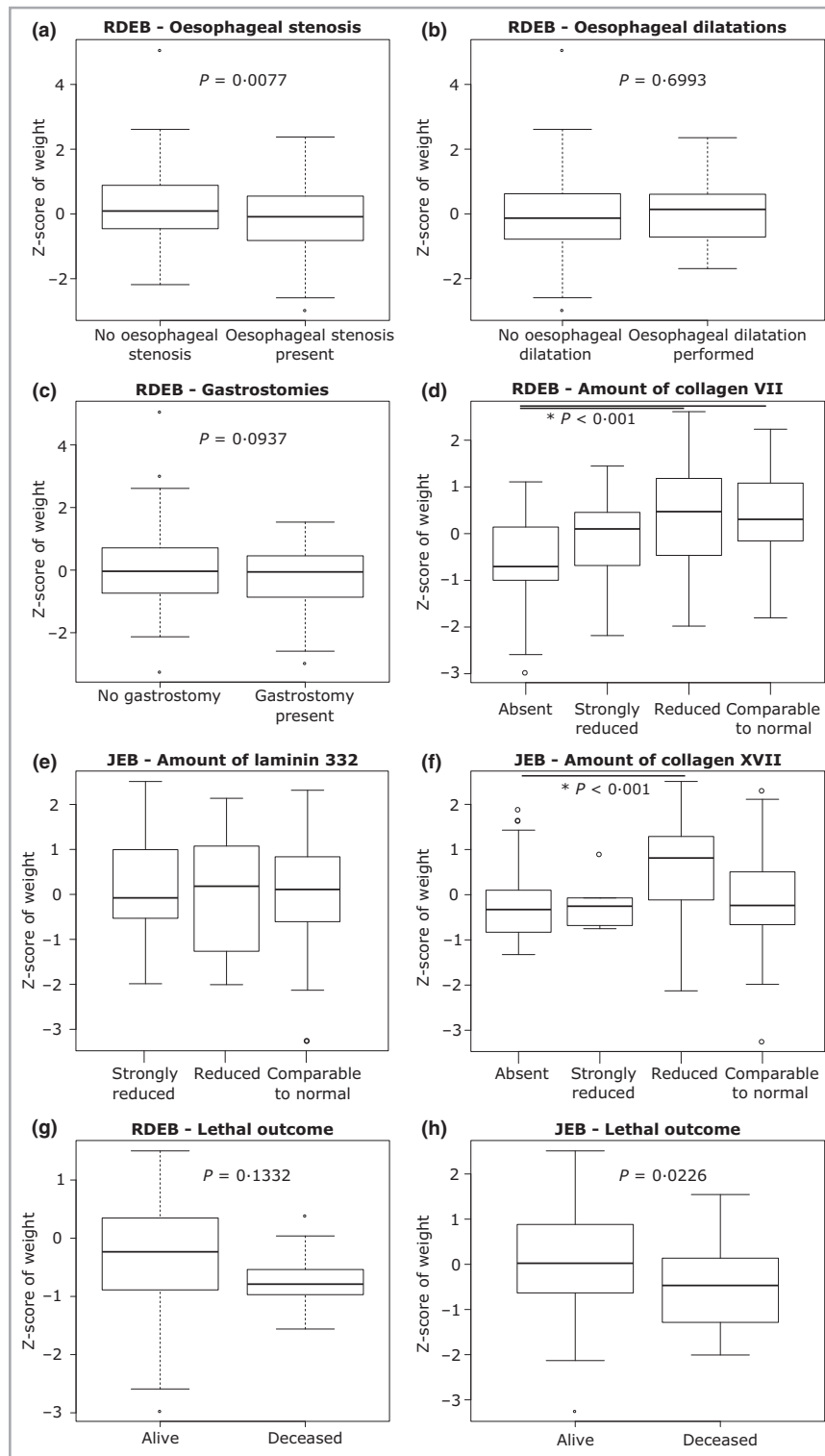


Fig 2. Correlation of epidermolysis bullosa (EB)-related clinical milestone events with weight Z-score. (a) Oesophageal stenosis in individuals with recessive-dystrophic EB (RDEB). (b) Oesophageal dilatations in individuals with RDEB. (c) Presence of a gastrostomy tube in RDEB. (d–f) Correlation between amount of affected protein in skin of patients with EB as determined by immunofluorescence staining and Z-score of weight. (d) Collagen VII in RDEB. Note that Z-score of weight is significantly lower when collagen VII is absent compared with reduced or normal collagen VII. (e) The amount of laminin 332 in the skin of patients with junctional EB (JEB)-generalized intermediate shows no significant correlation with Z-score for weight. (f) Collagen XVII in JEB. The correlation between absent and reduced collagen XVII ($P < 0.001$) is likely delusive, as no significant correlations between absence or strong reduction and normal collagen XVII were found and the sample size for this subgroup was low. (g) Lethal outcome in RDEB. (h) Lethal outcome in JEB.

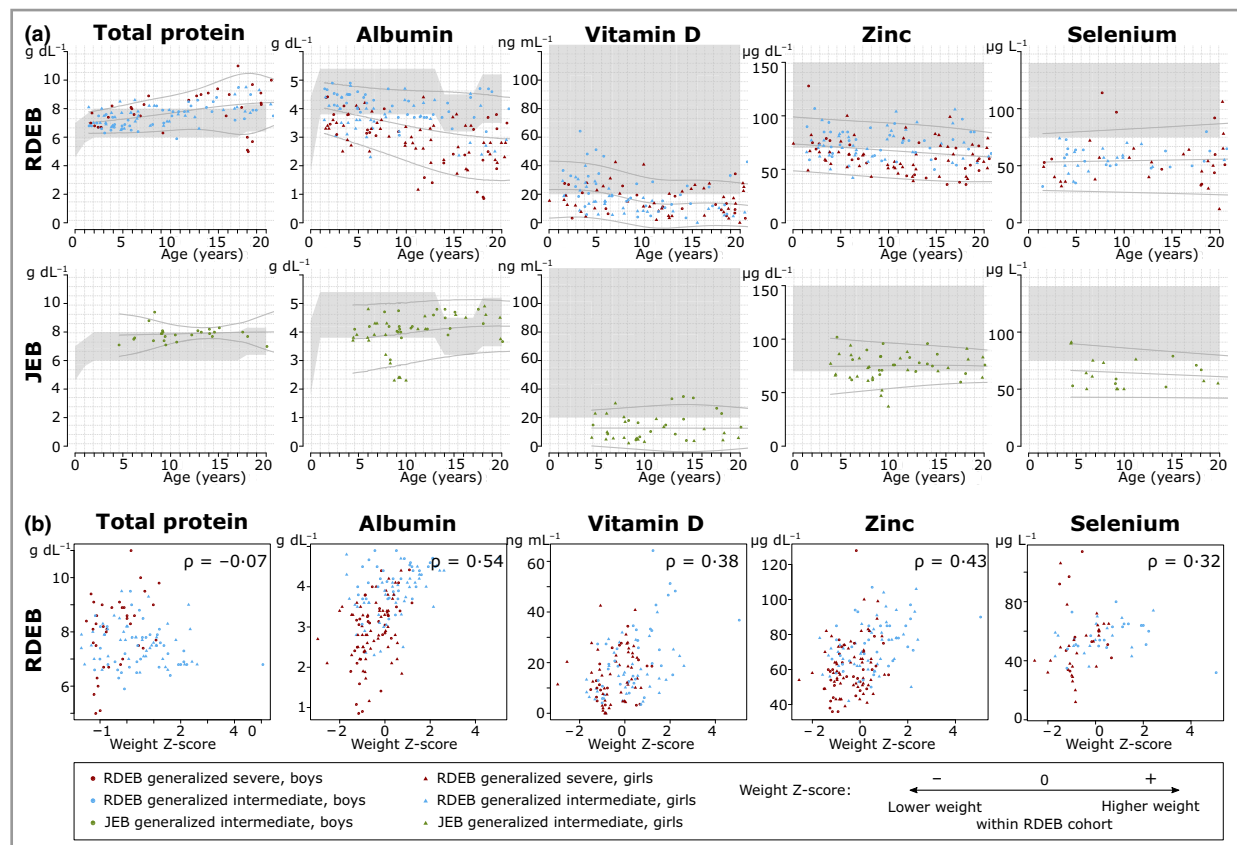


Fig 3. Nutritional parameters in recessive dystrophic epidermolysis bullosa (RDEB) and junctional EB (JEB)-generalized intermediate. (a) Laboratory findings of nutritional parameters in RDEB and JEB over the course of development (age 0–20 years). Individual measurements are marked with dots (RDEB-generalized severe, red; RDEB-generalized intermediate, blue; JEB-generalized intermediate, green). Normal ranges are depicted by grey areas. Trend lines for laboratory parameters were estimated using GAMLSS; lines represent fifth, 50th (median) and 95th percentiles. (b) Correlation of laboratory parameters of nutrition and Z-scores of weights of children and adolescents with RDEB. ρ = Spearman's rank-order correlation coefficient.

Ten individuals died during the observation period (Table 1): six with RDEB [median age 18.5 years (range 9.9–19.9)] and four with JEB [median age 11.3 years (range 5.7–13)]. The deceased children with JEB were particularly light (P3 in one of four, and P15 in three of four) compared with other children with JEB (Fig. 2g, h).

Children originating from war zones or countries with resource-limited settings were lighter than German children [significant for children from Syria ($P = 0.008$) and Turkey ($P = 0.009$), and nonsignificant for those from Iraq ($P = 0.016$)]. Impact of age at migration to Germany was not assessed in this study.

Growth charts for children with RDEB and JEB generated from this cohort are available in Figures S1–S4 (see Supporting Information); confidence intervals are tabulated in File S1 (see Supporting Information).

Natural history of nutrient levels and anaemia

Albumin levels were low in 56% of patients with RDEB and 22% of those with JEB, while total serum protein was

normal or elevated (Fig. 3a). Vitamin D deficiency was common both in RDEB and JEB (67% and 76%, respectively). Zinc and selenium deficiencies were common in RDEB (55% and 94%, respectively) and JEB (32% and 75%, respectively).

Anaemia was present in 91% of children with RDEB and 75% with JEB from the second year of life onwards (Fig. 4a, Table 2). With age, haemoglobin levels decreased further in RDEB, but improved towards adulthood in JEB (Fig. 2a). Serum iron levels, ferritin and transferrin saturation were below normal in half of children with RDEB aged 2–10 years and in > 80% of those aged > 10 years (Fig. 4a, Table 2).

Nutritional supplements were recommended in case of deficiencies and followed the general national recommendations for the age. In case of gastrointestinal side-effects, iron dosage was lowered. Intravenous administration of iron was recommended in severe anaemia and ineffective oral supplementation. Adherence to treatment was not assessed.

Significant positive correlations were found between weight Z-scores in RDEB and the levels of haemoglobin, albumin,

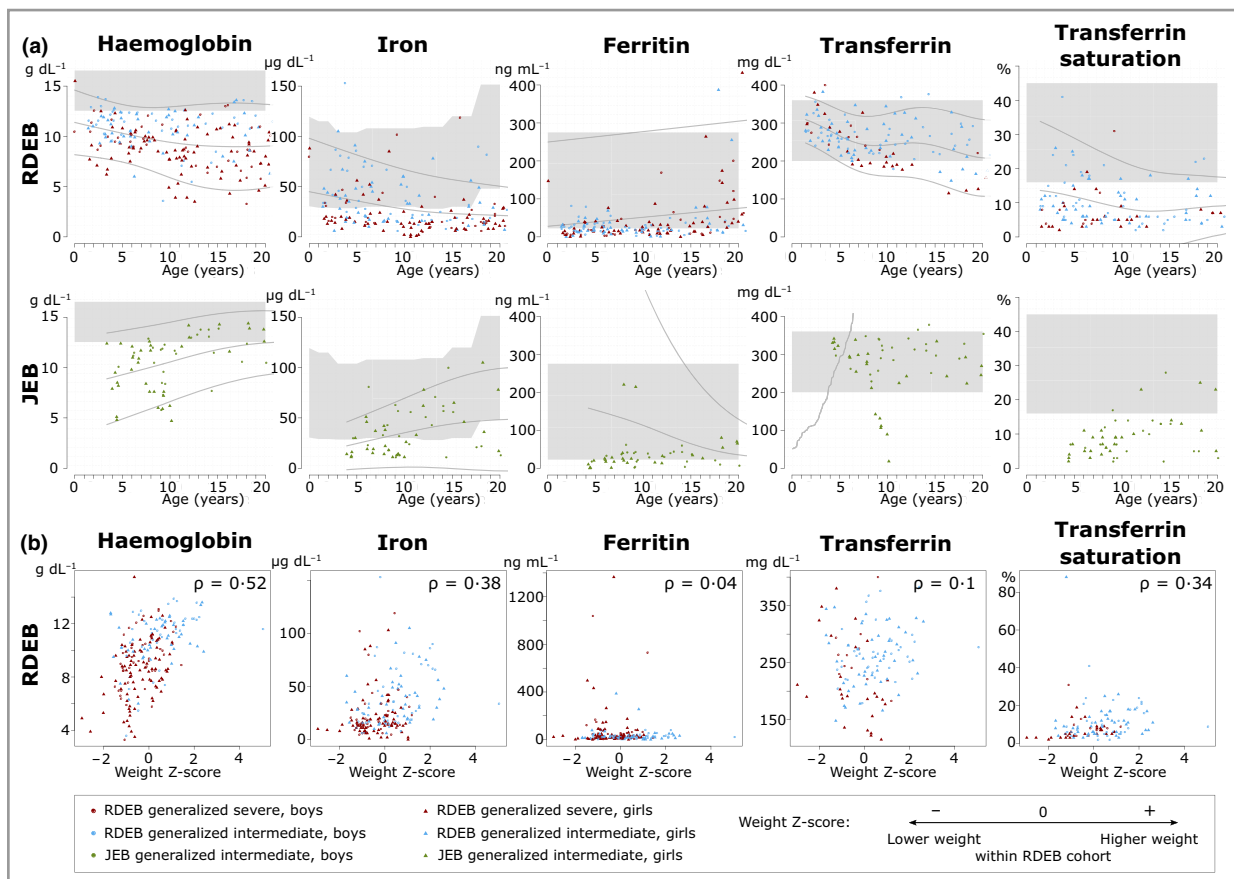


Fig 4. Anaemia in recessive dystrophic epidermolysis bullosa (RDEB) and junctional EB (JEB)-generalized intermediate. (a) Laboratory findings of anaemia parameters in RDEB and JEB over the course of development (age 0–20 years). Individual measurements are marked with dots (RDEB-generalized severe, red; RDEB-generalized intermediate, blue; JEB-generalized intermediate, green). Normal ranges are depicted by grey areas. Trend lines for laboratory parameters were estimated using GAMLSS; lines represent fifth, 50th (median) and 95th percentiles. (b) Correlation of laboratory parameters of anaemia and Z-scores of weights of children and adolescents with RDEB. ρ = Spearman's rank-order correlation coefficient.

vitamin D, zinc and iron ($P < 0.001$) (Figs 3b, 4b). There were no significant correlations between RDEB weight Z-scores and protein, TSH, transferrin, ferritin or selenium level. Laboratory parameters did not correlate with weight Z-scores in JEB.

Factors influencing growth: inflammation

Inflammation is an indicator of wound burden in EB, reflecting bacterial colonization and wound healing processes. Elevated CRP was present in 77% of measurements in RDEB (mean 52.8 ± 48.0 mg dL⁻¹) and 42% in JEB (mean 18.1 ± 33.5 mg dL⁻¹) (Table 2). In RDEB, CRP levels increased with age (Fig. 5a) and were significantly higher in individuals with severe vs. intermediate subtypes (Fig. 5b). Leucocyte counts were increased in 58% of measurements in RDEB and 36% in JEB. Elevated IgG levels were common in RDEB (72%) and JEB (73%), while IgA was more frequently elevated in RDEB (60%, compared

with 41% in JEB). No deviations in IgM levels were seen (Fig. 5a, Table 2). Elevated CRP and IgA – and thus inflammation – showed a significant negative correlation with weight Z-score in RDEB ($P < 0.001$) (Fig. 5c), but not in JEB. Leucocyte counts showed no correlation with weight in either RDEB or JEB.

Factors influencing growth: amount of affected protein in the skin

To assess whether growth is linked to collagen or laminin gene mutations, we correlated the amount of protein in the skin with weight Z-scores. Children with RDEB and absent collagen VII (clinical subtype generalized severe) showed a significantly lighter weight than those with reduced or near-to-normal collagen VII (clinical subtype generalized intermediate) (Fig. 2d). No correlations were found between amount of collagen XVII and laminin 332 in the skin and weight in JEB (Fig. 2e, f).

Table 2 Laboratory findings in children with epidermolysis bullosa (EB)

Parameter	Normal range	Children with RDEB		Children with JEB-generalized intermediate	
		Mean ± SD	Median (range)	Mean ± SD	Median (range)
Anaemia					
Haemoglobin (g L ⁻¹)	12.55–16.55	9.7 ± 2.23	10 (3.3–15.5)	11.1 ± 2.6	11.7 (4.7–15.7)
Reticulocytes (%)	4.8–16.4	17.8 ± 16.3	12 (0–112)	14.4 ± 12.9	11.4 (1.2–102)
Ferritin (μg L ⁻¹)	22.5–275	63.0 ± 140.8	27 (0–1365)	78.3 ± 198.5	29.5 (0–1281)
Transferrin (mg L ⁻¹)	200–360	241.7 ± 60.6	241 (115–400)	269.3 ± 66.3	283 (18–378)
Transferrin saturation, %	16–45	9.9 ± 8.85	7.5 (2–88)	10.4 ± 6.7	9 (2–30)
Iron (μg L ⁻¹)	26–151.5 ¹	27.6 ± 23.7	19 (0–153)	39.5 ± 26.3	33 (11–118)
Inflammation					
Leucocytes 1000 L ⁻¹	3.95–10.1	11.1 ± 3.5	10.5 (4–33.3)	9.5 ± 2.6	8.9 (2.4–15.8)
CRP (mg L ⁻¹)	< 3	52.8 ± 48.0	41 (0–193)	18.1 ± 33.5	7 (0–217)
IgA (mg L ⁻¹)	0–400 ²	470.7 ± 336.1	392 (12–1203)	311.4 ± 250.2	261 (74–1319)
IgG (mg L ⁻¹)	232–1600 ³	2444.3 ± 1309.8	2199.5 (635–6700)	1872.4 ± 597.9	1688 (1082–4070)
IgM (mg L ⁻¹)	0–259 ⁴	111.0 ± 44.1	102 (4.8–343)	124.7 ± 47.8	127 (50–277)
Nutrition and TSH					
Protein (g L ⁻¹)	4.4–8.3 ⁵	7.7 ± 1.0	7.7 (5–11)	7.9 ± 0.7	7.9 (6.3–9.4)
Albumin (g L ⁻¹)	3.2–5.4 ⁶	3.3 ± 0.9	3.4 (0.9–4.9)	4.0 ± 0.6	4.1 (2.3–5.1)
Zinc (μg L ⁻¹)	70–150	16.6 ± 12.0	13.7 (0–64.3)	12.8 ± 8.6	11.2 (0–34.9)
Selenium (μg L ⁻¹)	75–140	64.9 ± 15.7	63 (35–128)	73.6 ± 11.8	73 (37–102)
Vitamin D2/D3 (ng mL ⁻¹)	> 20	55 ± 18.1	55 (3.7–114)	61.9 ± 12.8	59 (43–91)
TSH (mIU L ⁻¹)	0.73–8.35 ⁷	2.2 ± 1.3	1.9 (0.3–7.4)	2.7 ± 1.5	2.3 (0.9–9.6)

RDEB, recessive dystrophic EB; JEB, junctional EB; CRP, C-reactive protein; TSH, thyroid-stimulating hormone. Reference ranges correspond to those depicted in Figure 2. Where applicable, reference ranges for male and female patients have been summarized for the statistical model. The following parameters have age-specific normal ranges. ¹Iron (μg dL⁻¹): 0–1 months, 30.5–119.5; 1–12 months, 26–117.5; 1–3 years, 29–115; 3–6 years, 28.5–104; 6–15 years, 30–108; 12–15 years, 28–109.5; 15–18 years, 30–120; ≥ 18 years, 48–151.5. ²IgA (mg dL⁻¹): < 12 months, 0–83; 1–3 years, 20–100; 3–6 years, 27–195, 6–9 years, 34–305, 11–13 years, 53–204; 13–15 years, 58–358; 15–19 years, 47–249; ≥ 19 years, 70–400. ³IgG (mg dL⁻¹): < 12 months, 232–1411; 1–3 years, 453–916; 3–6 years, 504–1465; 6–9 years, 572–1474; 9–11 years, 698–1560; 11–13 years, 759–1550; 13–15 years, 716–1711; 15–19 years, 549–1584; ≥ 19 years, 700–1600. ⁴IgM (mg dL⁻¹): < 12 months, 0–145, 1–3 years, 19–146; 3–6 years, 24–210; 6–9 years, 31–208; 9–11 years, 31–179; 11–13 years, 35–239; 13–15 years, 15–188; 15–19 years, 23–259; ≥ 19 years, 40–230. ⁵Total protein (g dL⁻¹): < 7 months, 4.4–7.6; 7–12 months, 5.1–7.3; 1–2 years, 5.6–7.5; 2–18 years, 6–8; ≥ 18 years, 6.4–8.3. ⁶Albumin (g dL⁻¹): < 14 years, 3.8–5.4; 14–18 years, 3.2–4.5; ≥ 18 years, 3.5–5.2. ⁷TSH (mIU L⁻¹): 3–12 months, 0.73–8.35; 1–6 years, 0.7–5.97; 6–11 years, 0.6–4.84; 11–20 years, 0.51–4.3.

RDEB, recessive dystrophic EB; JEB, junctional EB; CRP, C-reactive protein; TSH, thyroid-stimulating hormone. Reference ranges correspond to those depicted in Figure 2. Where applicable, reference ranges for male and female patients have been summarized for the statistical model. The following parameters have age-specific normal ranges. ¹Iron (μ g dL⁻¹): 0–1 months, 30.5–119.5; 1–12 months, 26–117.5; 1–3 years, 29–115; 3–6 years, 28.5–104; 6–15 years, 30–108; 12–15 years, 28–109.5; 15–18 years, 48–151.5. ²IgA (mg dL⁻¹): < 12 months, 0–83; 1–3 years, 20–100; 3–6 years, 27–195; 6–9 years, 34–305; 9–11 years, 53–204; 11–13 years, 58–358; 13–15 years, 47–249; 15–19 years, 61–348; > 19 years, 70–400. ³IgG (mg dL⁻¹): < 12 months, 232–1411; 1–3 years, 453–916; 3–6 years, 504–1465; 6–9 years, 572–1474; 9–11 years, 698–1560; 11–13 years, 759–1550; 13–15 years, 716–1711; 15–19 years, 549–1584; > 19 years, 700–1600. ⁴IgM (mg dL⁻¹): < 12 months, 0–145; 1–3 years, 19–146; 3–6 years, 24–210; 6–9 years, 31–208; 9–11 years, 31–179; 11–13 years, 35–239; 13–15 years, 15–188; 15–19 years, 23–259; > 19 years, 40–230. ⁵Total protein (g dL⁻¹): < 7 months, 4.4–7.6; 7–12 months, 5.1–7.3; 1–2 years, 5.6–7.5; 2–18 years, 6–8; > 18 years, 6.4–8.3. ⁶Albumin (g dL⁻¹): < 14 years, 3.8–5.4; 14–18 years, 3.2–4.5; > 18 years, 3.5–5.2. ⁷TSH (mIU L⁻¹): 3–12 months, 0.73–8.35; 1–6 years, 0.7–5.97; 6–11 years, 0.6–4.84; 11–20 years, 0.51–4.3.

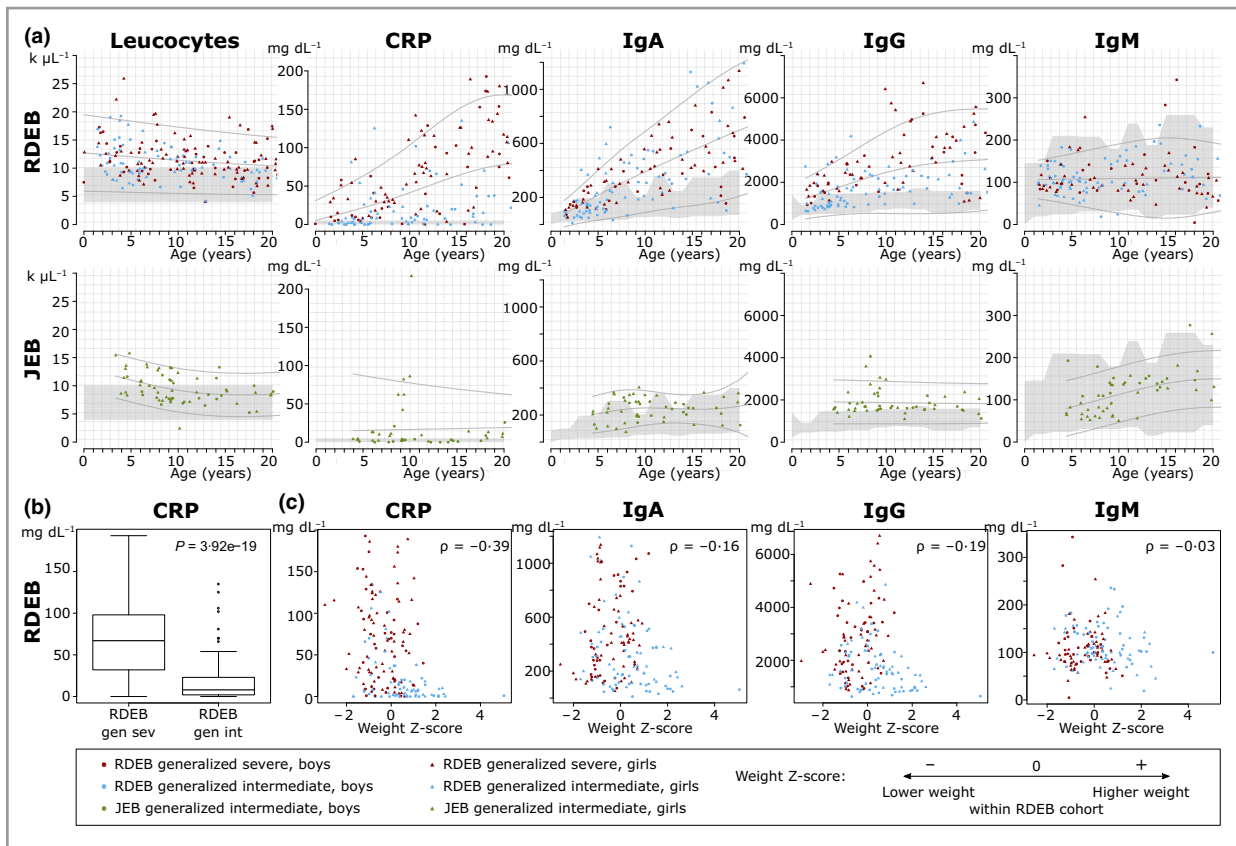


Fig 5. Inflammation in recessive dystrophic epidermolysis bullosa (RDEB) and junctional EB (JEB)-generalized intermediate. (a) Laboratory findings of inflammation parameters in RDEB and JEB over the course of development (age 0–20 years). Individual measurements are marked with dots (RDEB-generalized severe, red; RDEB generalized intermediate, blue; JEB generalized intermediate, green). Normal ranges are depicted by grey areas. Trend lines for laboratory parameters were estimated using GAMLSS; lines represent fifth, 50th (median) and 95th percentiles. (b) Boxplot of C-reactive protein (CRP) values measured in individuals with RDEB-generalized severe (gen sev) (left) and RDEB-generalized intermediate (gen int) (right). (c) Correlation of laboratory parameters of inflammation and Z-scores of weights of children and adolescents with RDEB. ρ = Spearman's rank-order correlation coefficient. Not shown: leucocytes.

Discussion

This study was designed to clarify the natural history of growth and anaemia in children with RDEB and JEB. In our cohort, > 50% of children with RDEB showed wasting and/or stunting, and > 80% of young adults with RDEB were underweight. Growth delay begins in the second year of life and is first visible in stagnating weight gain. This time frame suggests a correlation with the shift from milk and baby foods to solid foods, which are more likely to provoke swallowing difficulties due to mucosal involvement. Height development in RDEB is impaired later, confirming findings from a smaller cohort.²⁵ In JEB, where mucosa is less affected,²⁶ wasting and stunting are less common, but those children who died were particularly light,²⁷ underlining the predictive value of anthropometric measures and the need for close follow-up. Weight predicted EB growth patterns more specifically than did BMI, but the WHO weight reference used for comparison is given only up to the age of 10 years.

Owing to the rarity of this genetic disease, the relatively small sample size leads to a less smooth appearance of growth

curves compared with WHO charts. The heterogeneous mix of ethnicities within our cohort is a result of different migration waves over the last decades and reflects the current population in Germany, but socioeconomic^{28,29} and genetic³⁰ influences are possible. The growth charts obtained from our EB cohort can help to assess time points for nutritional interventions.

Nutritional compromise is regarded as the main factor contributing to underweight in RDEB.² We found that hypalbuminaemia, a feature previously reported in EB,^{25,31,32} correlated significantly with low weight in RDEB. As average protein and energy intake are well below general recommendations in children with RDEB^{3,9,25} while protein turnover is increased because of constant wound healing,³³ the body is driven into an autocatabolic state.³⁴ Although increasing oral intake is mostly futile,^{25,35} protein and energy supply can be increased by enteral feeding via gastrostomy.^{8,36} To add to the limited patient numbers in the literature,^{6,8,9} we have reported on the outcome of children with gastrostomies in our cohort. Gastrostomy insertion beyond the age of 8.5 years did not improve weight percentile rank in our patients. Whereas the

literature recommends gastrostomy before 10 years of age,⁹ our data suggest considering an earlier time point.

Several micronutrients were previously described as diminished in EB.^{32,37} Our data clearly show that deficiencies of vitamin D, zinc and selenium are already present in the second year of life, and in spite of recommended supplementation. Low weight in RDEB correlates significantly with low levels of zinc and vitamin D. Serum levels of trace elements can be low in inflammatory states^{38,39} and zinc levels can be falsely low in hypalbuminaemia. Zinc is an essential cofactor for a multitude of biochemical reactions, including those for wound healing.³⁹ Vitamin D has, next to its key role in bone metabolism, immunomodulatory and anti-inflammatory properties,^{40,41} and its deficiency possibly contributes to anaemia.⁴² Supplementation of Vitamin D3 is recommended for all children during the first year of life to prevent rickets.⁴³ Our results suggest that vitamin D supplementation should be continued throughout childhood in RDEB and JEB.

Anaemia is a severe complication of both RDEB and JEB^{5,11} and is especially difficult to treat. We have shown that anaemia and iron deficiency are already present in the second year of life, correlate with low weight and worsen with age in RDEB. This is in line with the general deterioration of nutrition and disease progression and more common mucosal involvement in RDEB, whereas in JEB anaemia improved and weight development was near to normal. In our centre, we recommend oral or intravenous iron administration in cases of iron-deficiency anaemia, but iron supplementation is unpopular with patients as it increases gastrointestinal symptoms such as constipation and stomach pain.⁴⁴ The optimal strategy for treating anaemia in EB is still under discussion,¹¹ but should involve ongoing supplementation of iron and cofactors for haemoglobin synthesis, minimizing blood loss from wounds and reducing inflammation.

Inflammation, arising from constant wounds and enhanced by bacterial colonization,^{45,46} is an important disease determinant in EB. We used CRP, leucocytes and immunoglobulins as measures of inflammation and thus disease activity. In RDEB, inflammatory markers are constantly increased and significantly correlate with low weight. Anecdotally, systemic anti-inflammatory treatment can lead to reduction of wound burden and improvement of anaemia in JEB.⁴⁷ This underlines the complex interactions of inflammation, anaemia and nutrition, highlighting that these aspects must be regarded and treated as a whole.

We further asked whether intrinsic, genetic factors influence growth in EB. As RDEB is caused by absence or reduction of collagen VII in the skin,⁴⁸ and increase of collagen VII led to clinical improvement in an RDEB mouse model,⁴⁹ it was of interest to correlate its abundance with the affected individuals' growth. Our results show that any amount of collagen VII, compared with complete absence, is associated with a better outcome regarding weight. It is still unsolved whether this finding is because skin and mucous membranes are more severely affected, or whether as yet unidentified collagen VII-dependent processes play an additional role, such as malabsorption caused by lack of collagen VII in the intestinal mucosa.

Our results suggest that nutritional intervention in EB should start before the age of 2 years, prior to stagnation of weight gain. Weight is a straightforward marker for prognosis and therapy planning; therefore we propose weight measurements for children with EB every 3 months. Our growth charts can serve as tools to assess growth of children with severe EB in daily practice. The profound nutritional deficits revealed in our cohort show that nutritional supplementation should always be given. Risk of overdosage in children with severe EB appears low. Our data suggest a decreased need for blood sampling, namely every 12 or even 24 months, which will minimize painful procedures and facilitate care in resource-poor settings. Prospective studies are needed to assess the effect of nutritional interventions, identify the optimal EB adapted supplementation dosages, and address the necessity for dose escalation in severe disease.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

File S1. Tabulated confidence intervals for growth charts of our cohort.

Fig S1. Combined height-for-age and weight-for-age growth charts for boys with recessive dystrophic epidermolysis bullosa compared with those of healthy children, age range 0–18 years.

Fig S2. Combined height-for-age and weight-for-age growth charts for girls with recessive dystrophic epidermolysis bullosa compared with those of healthy children, age range 0–18 years.

Fig S3. Combined height-for-age and weight-for-age growth charts for boys with junctional epidermolysis bullosa generalized intermediate compared with those of healthy children, age range 0–18 years.

Fig S4. Combined height-for-age and weight-for-age growth charts for girls with junctional epidermolysis bullosa generalized intermediate compared with those of healthy children, age range 0–18 years.

Powerpoint S1 Journal Club Slide Set.