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ORIGINAL ARTICLE

Long-term effects of growth hormone (GH) replacement therapy on hematopoiesis in a large cohort of children with GH deficiency

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Abstract The aim of our prospective case-control study was to evaluate long-term effects of GH replacement therapy on erythrocytes parameters, leukocytes, and platelets numbers in a large cohort of children with isolated GH deficiency (GHD). Hemoglobin (Hb) concentration, hematocrit (Hct), mean corpuscular volume, mean corpuscular hemoglobin, red cell distribution width, number of erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes and platelets, ferritin, and C-reactive protein were evaluated in 85 children with isolated GHD (10.20 ± 3.50 years) before and annually during the first 5 years of GH replacement therapy and in 85 healthy children age and sex comparable to patients during 5 years of follow-up. Compared with controls, GHD children at study entry showed lower Hb (-1.18 ± 0.87 vs. -0.40 ± 0.90 SDS, $p < 0.0001$), red cells number (-0.24 ± 0.81 vs. 0.25 ± 1.14 SDS, $p < 0.0001$), and Hct (-1.18 ± 0.86 vs. -0.68 ± 0.99 SDS, $p < 0.0001$). Twelve GHD patients (14 %) showed a normocytic anemia. GH therapy was associated with a significant increase in Hb, Hct, and red cells number which became all comparable to controls within the first 2 years of treatment. Moreover, hemoglobin levels normalized in all anemic GHD patients after 5 years of therapy. No difference between patients and controls was found in leukocytes and platelets numbers neither at baseline nor during the study. GHD in childhood is associated with an impairment of erythropoiesis which causes a normocytic anemia in a considerable percentage of patients. GH replacement therapy exerts a beneficial effect leading to a significant increase of erythrocytes parameters and recovery from anemia. Neither GHD nor GH replacement treatment exerts effects on leukocytes or platelets numbers.

Keywords GH · GH deficiency · Hemochrome · Anemia · Platelets · Leukocytes

Introduction

Growth hormone (GH) in addition to promote linear growth in childhood exerts a key role in the regulation of several metabolic processes [1] either directly or through insulin-like growth factor 1 (IGF-1). GH and IGF-1 influence hematopoiesis through specific receptors on hematopoietic cell lines. Bone marrow is considered an important target of GH/IGF-1 action [2, 3]; indeed, GH and IGF-1 modulate hematopoiesis directly through their proliferative and anti-apoptotic effects and indirectly by regulating cytokine production [3, 4].

Several studies in GHD mice documented reduced peripheral erythrocytes, leukocytes, and platelets which significantly increased after GH replacement [5]. In addition, normocytic anemia, restored by IGF-1 administration, has been reported in hypophysectomised rats [6].

Human studies revealed that GHD in both adults and children can be associated with an impairment of erythropoiesis leading to normocytic anemia [7–11], reduced red cell mass [12, 13], or reduced erythroid precursor cells number [14], which improved after GH treatment [7–14].

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Furthermore, GH exerts a modulatory action on thymic cells proliferation, cytokines production, and T-cells migration [15]. Moreover, GH therapy has been associated with increased neutrophils count [16] and myeloid precursors number [14], while other studies did not document effects of GH treatment on leukocytes count [10, 17]. Data in children are scanty, but GH seems to have no effects on leukocytes number [10, 13, 18–20].

GH promotes the differentiation of megakaryocytes and platelets production as documented in recent in vitro studies [21]; however, no effects of GHD and GH therapy on platelets count have been so far demonstrated in human studies.

We designed this prospective case-control study in order to evaluate long-term effects of GH replacement therapy on haemopoiesis in a large cohort of GHD children.

Subjects and methods

Patients and controls

Eighty-five prepubertal children with isolated GHD (55 males and 30 females) aged 10.20 ± 3.50 years were enrolled in the study. The diagnosis of GHD was based on clinical and auxological criteria and on GH peak <10 µg/l after two stimulation tests [22]. Patients with multiple hormones deficiency were excluded from the study to avoid the influence of other hormone deficiencies on the hematopoietic system. Magnetic resonance imaging (MRI) of the hypothalamus-pituitary region was normal in 55 patients, while 25 patients had pituitary hypoplasia, 4 pituitary cyst, and 1 lipoma.

No patient had received GH replacement before entering the study. Chronic diseases, iron deficiency, and thalassemia trait which could represent confounding factors were considered as exclusion criteria.

Eighty-five healthy children sex and age comparable to the patients were enrolled in the study as controls; they were selected among children referred to our clinic for short stature or thyroid assessment. After a complete assessment, they were found to have familial short stature or to be healthy euthyroid children.

Study protocol

At study entry, all subjects underwent measurement of weight and height, and BMI was then calculated. Anthropometric measures were normalized for sex and age according to Cacciari standards [23]. Serum levels of IGF-1 and complete blood parameters consisting of measurement of hemoglobin (Hb) concentration, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), number of erythrocytes, leukocytes, neutrophils, lymphocytes, monocytes, and platelets were evaluated. Erythrocytes parameters were normalized for sex and age according to reference values [24]. Ferritin and C-reactive protein (CRP) were measured to evaluate iron metabolism and inflammation status.

After diagnosis, GHD children started GH replacement therapy at a dose of 25 mcg/kg/day. All parameters were evaluated annually for 5 years in GHD patients and controls.

Informed parental consent for participation in the study was obtained for patients and controls and the study was authorized by the Hospital Ethical Research Committee.

Assay

GH levels were measured by immunoradiometric assay (IRMA) using the kit HGH-CTK-IRMA Sorin, Saluggia, Italy. IGF-I levels were determined by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 2000 Siemens Healthcare Diagnostics Inc., New York, USA). Complete blood counts were performed by an automated hematology analyzer (Siemens Advia 2120).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed by the software SPSS/PC 20.0 (SPSS Inc, Chicago II).

Comparison between GHD patients and healthy controls was performed by Student's *t* test or Wilcoxon test as appropriated. Comparison of patients and controls longitudinally during the study was performed by one-way analysis of variance (ANOVA) for repeated measures. Pearson's correlation coefficient was used to evaluate the relationship between variables.

Probability values less than or equal to 0.05 were considered statistically significant.

Results

Clinical features at study entry in GHD patients and controls are reported in Table 1.

As expected, at study entry, height (−2.33 ± 0.85 vs. −0.77 ± 1.29 SDS, $p < 0.0001$) and IGF-1 (−1.80 ± 0.76 vs. −0.12 ± 1.06 SDS, $p < 0.0001$) levels were significantly lower in GHD patients compared to controls. No difference was observed in BMI (−0.49 ± 1.23 vs. −0.17 ± 1.18 SDS).

One year of GH replacement therapy was associated with a significant increase in height (−1.80 ± 0.72 SDS, $p < 0.0001$; Δ-height +0.52 SDS) and IGF-1 levels

Table 1 Clinical features at study entry in GHD patients and controls

	GHD patients	Controls	*p*
Male/female	55/30	55/30	NS
Age (years)	10.20 ± 3.50	9.34 ± 4.60	NS
Height (SDS)	−2.33 ± 0.85	−0.77 ± 1.29	<0.0001
BMI (SDS)	−0.49 ± 1.23	−0.17 ± 1.18	NS
IGF-1 (SDS)	−1.80 ± 0.76	−0.12 ± 1.06	<0.0001

(0.43 ± 1.24 SDS, $p < 0.0001$), the latter becoming comparable to controls (0.02 ± 1.11 SDS). Thereafter, IGF-1 levels remained stable, while height continued to increase significantly becoming comparable to the controls after 5 years of GH treatment (−1.23 ± 1.00 vs. −0.75 ± 1.08 SDS; Δ-height +1.10 SDS).

Erythropoiesis

At baseline, GHD patients compared to controls showed significantly lower values of Hb (SDS −1.18 ± 0.87 vs. −0.40 ± 0.90, $p < 0.0001$) (12.50 ± 0.80 vs. 13.30 ± 0.90 g/dl, $p < 0.0001$), red cells number (SDS −0.24 ± 0.81 vs. 0.25 ± 1.14, $p < 0.0001$) (4.60 ± 0.36 vs. 4.95 ± 0.34 × 10^6/mcl, $p < 0.0001$), and Hct (SDS −1.18 ± 0.86 vs. −0.68 ± 0.99, $p < 0.0001$) (36.60 ± 1.92 vs. 37.80 ± 1.95 %, $p < 0.0001$). No difference was observed in MCV, MCH, and RDW. Red cells parameters expressed in SDS in GHD patients and controls throughout the study are reported in Table 2 and depicted in Fig. 1.

A subset of 12 GHD children (14 %) showed normocytic anemia, with hemoglobin levels below −2 SD (−2.75 ± 0.65 SDS), whereas within the control group only 2 anemic children (2 %) were identified ($\chi^2 = 7.78$, $p < 0.01$). Anemic GHD patients showed a more severe GH deficiency as documented by lower IGF-1 levels compared with not-anemic GHD patients (−2.91 ± 0.28 vs. −1.58 ± 0.63 SDS, $p < 0.0001$).

Red cells parameters, leukocytes, and platelets counts over the study period in anemic GHD patients compared to matched controls are reported in Fig. 2.

GH replacement therapy had a positive effect on red cells parameters, which became all comparable to controls at the end of the study (Table 2; Fig. 1). In addition, during the 5 years, hemoglobin levels normalized in all anemic GHD patients (Fig. 2).

A positive correlation was observed between IGF-1 levels and Hb ($r = 0.69$, $p = 0.001$), red cells number ($r = 0.16$, $p = 0.001$), Hct ($r = 0.18$, $p < 0.0001$).

CRP and ferritin levels were in the reference range in all children and were comparable between patients and controls during the study (Table 2).

Leucopoiesis

At study entry, no difference was detected in total leukocytes number or in neutrophils, lymphocytes, and monocytes numbers between GHD children and healthy controls. No difference in these parameters between the two groups was found throughout the study. However, both GHD patients and controls showed a significant decrease in white cells count which resulted comparable between the two groups. Details on leukocytes number are reported in Table 2 and Fig. 1.

Thrombopoiesis

No difference was found between GHD patients and controls in platelets count either at baseline or during the whole study. A significant reduction in this parameter was observed during the study in both the groups and resulted comparable between patients and controls (Table 2; Fig. 1).

Discussion

Our results suggest that untreated GHD in children is associated with abnormalities in erythropoiesis characterized by a reduction in Hb, Hct, and red cells number, although these parameters remain in the normal range for age in most patients. Mild anemia has been documented in 14 % of GHD children at baseline. Long-term GH replacement treatment was associated with an improvement in red cells parameters in the absence of any other treatment, also in anemic patients, which became all comparable to age- and sex-matched controls. Neither GHD nor GH replacement therapy had effects on leucopoiesis and thrombopoiesis; indeed leukocytes and platelets counts were comparable in GHD patients and controls during the whole period of the study.

Studies evaluating red cells parameters in GH deficiency have yielded contrasting results. Indeed, some studies reported normocytic anemia in untreated GHD adults [8, 10] and children [7, 9–11], while others reported low hematopoietic precursor cells but not anemia in GHD [14]. However, most authors reported a beneficial effect of GH replacement therapy on red cells with an increase of Hb levels [7–11, 13, 17], red cells mass [12, 13], and hematopoietic precursors number [14].

GH/IGF-1 axis has been thought to influence erythropoiesis both directly and indirectly. Indeed, GH receptors are expressed on hematopoietic progenitor cells [13] and there is evidence that GH modulates gene expression and stimulates proliferation in hematopoietic cells through Epo-independent pathways [13]. Moreover, GH stimulates

Table 2 Red cells parameters in GHD patients and controls at baseline and during the follow-up

	Baseline	1 year	2 years	3 years	4 years	5 years
Hb (SDS)						
GHD patients	−1.18 ± 0.87[a]	−0.94 ± 0.83[a,b]	−0.85 ± 0.86[a,b]	−0.72 ± 0.89[b]	−0.50 ± 0.90[b]	−0.47 ± 0.94[b]
Controls	−0.40 ± 0.90	−0.42 ± 1.08	−0.40 ± 0.70	−0.45 ± 0.96	−0.39 ± 0.88	−0.43 ± 0.87
Red cells (SDS)						
GHD patients	−0.24 ± 0.81[a]	−0.12 ± 0.94[a]	−0.06 ± 0.92	0.01 ± 0.90	0.23 ± 0.94[b]	0.26 ± 0.91[b]
Controls	0.25 ± 1.14	0.26 ± 1.20	0.23 ± 1.24	0.21 ± 1.24	0.23 ± 1.22	0.24 ± 1.26
Hct (SDS)						
GHD patients	−1.18 ± 0.86[a]	−1.03 ± 0.83[a]	−0.85 ± 0.75[b]	−0.83 ± 0.74[b]	−0.73 ± 0.75[b]	−0.58 ± 0.80[b]
Controls	−0.68 ± 0.99	−0.69 ± 0.92	−0.65 ± 0.92	−0.69 ± 0.98	−0.67 ± 0.84	−0.69 ± 0.82
MCV (SDS)						
GHD patients	−1.06 ± 0.80	−1.08 ± 0.74	−0.95 ± 0.75	−0.85 ± 0.65	−0.89 ± 0.53	−0.86 ± 0.66
Controls	−0.97 ± 1.04	−1.04 ± 0.93	−1.02 ± 0.78	−0.87 ± 0.65	−0.94 ± 0.80	−0.87 ± 0.72
MCH (SDS)						
GHD patients	−0.63 ± 0.75	−0.62 ± 0.67	−0.60 ± 0.66	−0.58 ± 0.62	−0.54 ± 0.59	−0.50 ± 0.71
Controls	−0.58 ± 0.76	−0.60 ± 0.73	−0.62 ± 0.67	−0.61 ± 0.61	−0.58 ± 0.73	−0.56 ± 0.84
RDW (%)						
GHD patients	13.40 ± 1.01	13.44 ± 1.05	13.36 ± 1.07	13.35 ± 1.13	13.26 ± 1.02	13.14 ± 0.86
Controls	13.37 ± 0.91	13.41 ± 1.27	13.41 ± 1.36	13.38 ± 1.14	13.19 ± 0.72	13.28 ± 0.82
Leukocytes (10^3/mcl)						
GHD patients	7.27 ± 2.40	6.97 ± 2.05	6.73 ± 2.29	6.50 ± 1.64[b]	6.40 ± 1.50[b]	6.20 ± 1.71[b]
Controls	7.37 ± 2.01	7.13 ± 1.56	7.04 ± 2.36	6.74 ± 1.35	6.65 ± 1.26	6.51 ± 1.49[b]
Neutrophils (10^3/mcl)						
GHD patients	3.57 ± 1.71	3.52 ± 1.34	3.47 ± 1.90	3.43 ± 1.21	3.38 ± 1.11	3.34 ± 1.25
Controls	3.53 ± 1.50	3.52 ± 1.32	3.55 ± 1.87	3.48 ± 1.78	3.45 ± 1.69	3.39 ± 1.32
Lymphocytes (10^3/mcl)						
GHD patients	2.79 ± 0.80	2.70 ± 0.88	2.64 ± 0.83	2.47 ± 0.58[b]	2.51 ± 0.73[b]	2.39 ± 0.54[b]
Controls	2.92 ± 0.92	2.90 ± 0.59	2.76 ± 0.66	2.63 ± 0.56	2.68 ± 0.57	2.52 ± 0.68[b]
Monocytes (10^3/mcl)						
GHD patients	0.44 ± 0.20	0.42 ± 0.14	0.43 ± 0.14	0.42 ± 0.12	0.42 ± 0.13	0.41 ± 0.16
Controls	0.48 ± 0.20	0.44 ± 0.18	0.45 ± 0.16	0.43 ± 0.11	0.43 ± 0.12	0.42 ± 0.14
Platelets (10^{11}/l)						
GHD patients	3.13 ± 0.82	3.06 ± 0.85	2.83 ± 0.64[b]	2.66 ± 0.56[b]	2.66 ± 0.60[b]	2.58 ± 0.47[b]
Controls	3.14 ± 0.67	3.04 ± 0.64	2.86 ± 0.61[b]	2.71 ± 0.63[b]	2.71 ± 0.56[b]	2.63 ± 0.50[b]
CRP (mg/dl)						
GHD patients	0.34 ± 0.11	0.37 ± 0.21	0.32 ± 0.03	0.32 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
Controls	0.35 ± 0.17	0.36 ± 0.16	0.34 ± 0.11	0.33 ± 0.13	0.36 ± 0.18	0.32 ± 0.01
Ferritin (ng/ml)						
GHD patients	35.46 ± 13.26	32.86 ± 12.41	34.51 ± 11.98	33.31 ± 12.69	35.08 ± 12.91	34.92 ± 11.78
Controls	31.04 ± 14.39	33.42 ± 11.28	33.62 ± 13.88	34.81 ± 15.80	32.21 ± 12.26	33.47 ± 12.49

[a] $p < 0.05$ versus controls

[b] $p < 0.05$ versus baseline

erythropoietin (Epo) production [8, 20] and potentiates Epo action by binding Epo receptors [25]. IGF-1 also has a Epo-like action stimulating erythroid maturation and proliferation by acting on its own receptors expressed on erythroid precursors [10, 17]. Furthermore, GH stimulates IGF-1 production by hematopoietic progenitors themselves thus suggesting an autocrine/paracrine role of IGF-1 in hematopoietic system [13].

In our study, the link between GH/IGF-1 axis and red cells parameters is strengthened by the lower IGF-1 levels

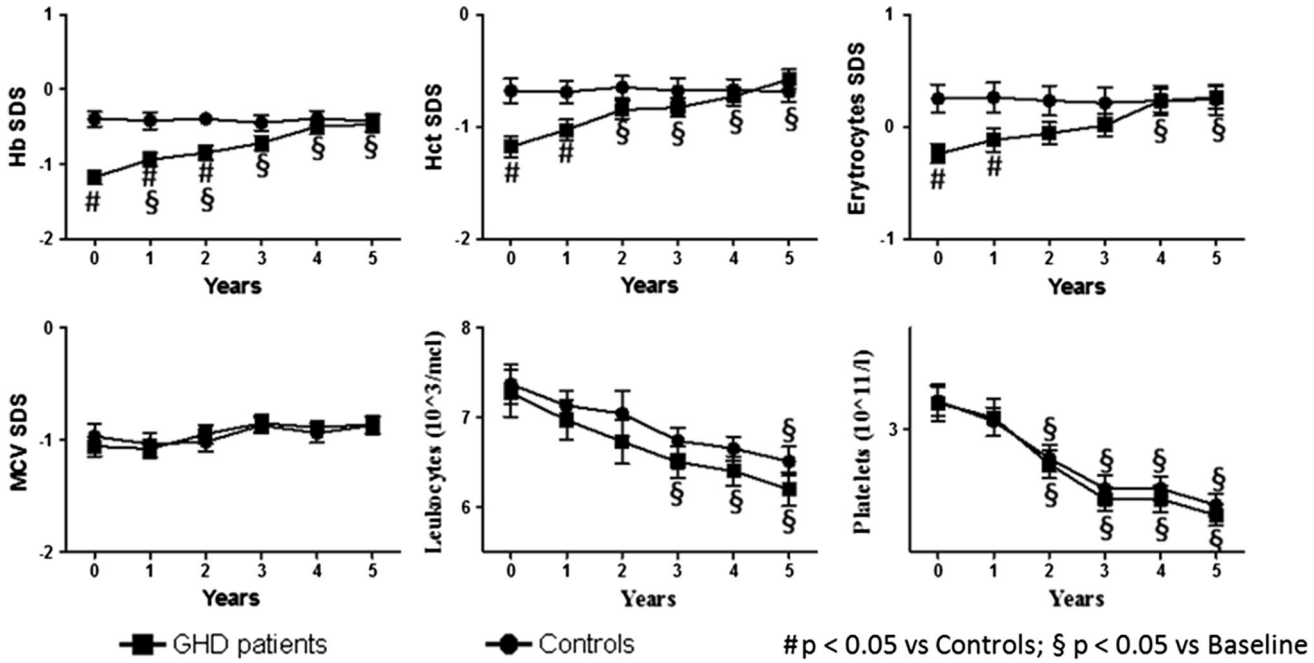

Fig. 1 Red cells parameters, leukocytes and platelets counts in GHD patients and controls

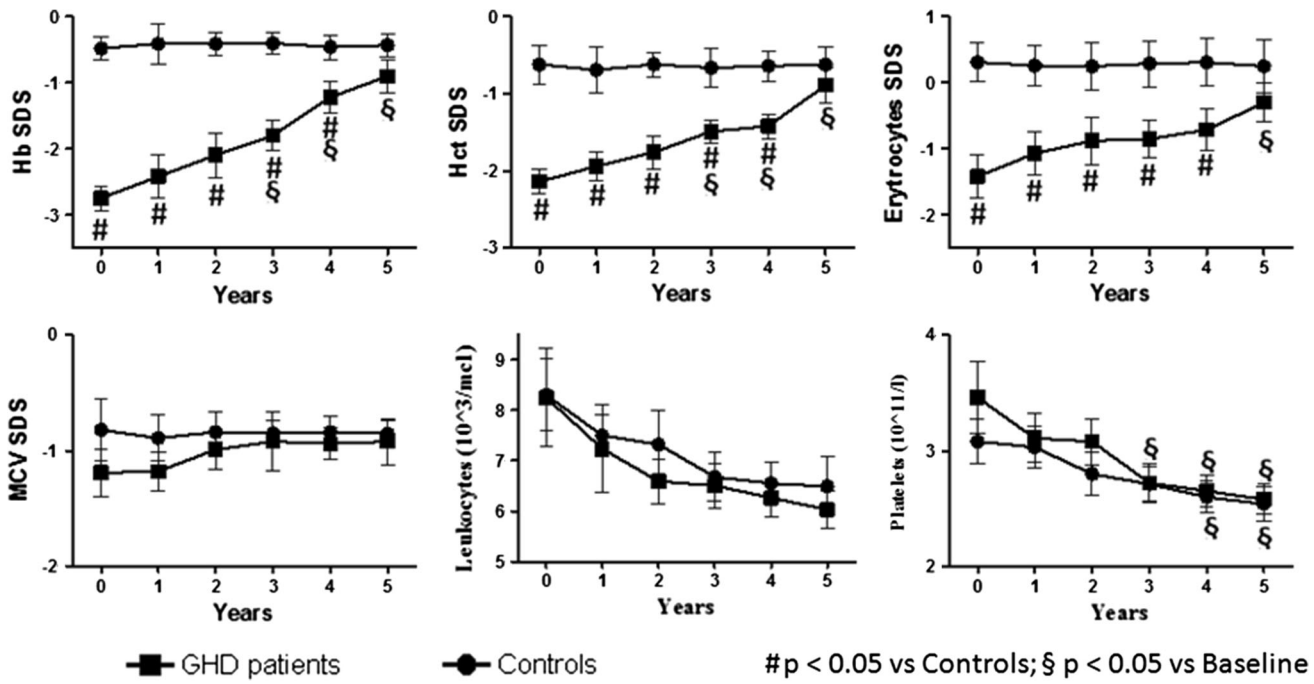

Fig. 2 Red cells parameters, leukocytes and platelets counts in anemic GHD patients and matched controls

documented in anemic GHD children and by the positive correlation detected between IGF-1 levels and hematological parameters already described in GHD patients [11, 17].

Although an improvement in red cells parameters was evident in the first year of treatment, their normalization was only observed after 2 years of GH, whereas IGF-1 levels normalized by the end of the first year of therapy. This finding suggests that metabolic effects of GH replacement therapy on erythropoiesis are not immediate and may take longer than time required to normalize IGF-1 levels. Similarly, the beneficial effect of GH replacement on metabolic parameters has been observed in several

studies only after 2 years of therapy in GHD children [26, 27].

To our knowledge, this is the first long-term case-control study evaluating hematopoiesis in a large cohort of GHD children. Even though red cells parameters have already been assessed in large series of GHD children [9, 11, 13], only two short-term studies enrolled a control group [7, 13]. Furthermore, we extended the evaluation to all red cells parameters and to inflammatory markers and iron balance in order to exclude any confounding factor influencing erythropoiesis, although we acknowledge that CRP and ferritin alone are not sufficient to completely exclude a condition of chronic inflammation in our patients.

We also investigated the effects of GHD and GH replacement therapy on white cells and platelets numbers. In vitro and in vivo studies have shown that GH influences immune systems by stimulating neutrophils differentiation and activation, modulating thymic microenvironment, increasing T-cells proliferation and cytotoxicity, promoting antibody synthesis by B-cells and regulating interleukins production [15, 28]. In keeping with these findings, withdrawal of GH replacement therapy in GHD adults is associated with impairment in thymus and T-cells function, which is restored by treatment restart [29]. Several studies in adults documented that GH replacement was associated with the reduction of monocyte hyperactivation [30], increase of neutrophils [16], and myeloid precursors number with no significant effects on peripheral cells [14], while others did not identify any effect of GH therapy on leukocytes count and function [10, 17, 31, 32].

Most studies in GHD children reported that neither GHD nor GH replacement have effect on leukocytes count [10, 13, 18, 19], but an impairment of phagocytic function in GHD children, which was improved by GH treatment, has been reported [19].

Our study confirms these results on a larger cohort of patients; indeed, the number of leukocytes, neutrophils, lymphocytes, and monocytes was comparable between GHD patients and healthy controls at study entry and during GH replacement therapy. The reduction in leukocytes during the follow-up was comparable between GHD patients and controls reflecting physiological changes during growth [24].

Although GH has been reported to promote megakaryocytes maturation and platelets production in vitro and GH receptors have been identified on human megakaryocytes at various differentiation stages [21], neither GHD nor GH therapy seems to have an impact on platelets count in adulthood and in childhood [10, 13, 17]. Accordingly to these previous findings, our results did not document any significant effect of GHD or GH treatment on platelet count.

A limitation of our study is that we only evaluated the effects of GHD and GH replacement on the number of leukocytes and platelets and we did not perform functional tests of these cells.

In conclusion, our study suggests that untreated GHD in childhood is associated with an impairment of erythropoiesis. GH replacement therapy exerts a beneficial effect leading to a significant increase of erythrocytes parameters. Neither GHD nor GH replacement treatment exerts effects on leukocytes and platelets numbers. However, whether GHD might be associated with a functional impairment of these cells should be further investigated.

Compliance with Ethical Standards

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

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