Low-dose non-steroidal anti-inflammatory drugs: a promising approach for the treatment of symptomatic bone marrow failure in Ghosal hematodiaphyseal dysplasia

Ghosal hematodiaphyseal dysplasia (GHD) is a rare autosomal recessive disease characterized by severe anemia and painful long-bone diaphyseal cortical endosteal hypertrophy.[1] Some 40 cases have been reported, mostly in childhood. The disease results from biallelic deleterious variants in the *TBXAS1* gene, disrupting TXAS protein function, causing thromboxane A2 pathway blockage and arachidonic acid metabolite accumulation.[2,3] Standard care involves systemic corticosteroids, effectively alleviating hematopoietic and bone disorders. However, patients often require long-term corticosteroids, leading to iatrogenic complications.

Based on the thromboxane A2 pathway dysregulation, Brown *et al.*[4] reported for the first time the good efficacy of cyclo-oxygenase (COX) 1 and 2 inhibition with non-steroidal anti-inflammatory drugs (NSAID) in two patients. Based on a similar hypothesis, four other non-related patients with GHD were treated in France from June 2019 with low-dose NSAID.

The French bone-marrow failure observatory (study's ethic committee approval CLEA-2023-#312) was consulted to identify patients with biallelic pathogenic *TBXAS1* variants, who underwent treatment with low doses of aspirin or NSAID.

During screening, four unrelated patients were identified among 1,857 patients included in the observatory. The characteristics of these patients and their disease are detailed in Table 1. Our four patients are male, with no medical history other than GHD. Patients #1, #3 and #4 had severe transfusion-dependent anemia in early childhood. Patients #1 and #3 achieved spontaneous red blood cell (RBC) transfusion independence without treatment for over 20 years. Patient #4 required long-lasting steroid treatment, to which he had a partial response and required occasional RBC transfusions. Patient #2 had no hematologic issues until the age of 24 years.

In the months preceding treatment with NSAID, all patients had severe anemia (median hemoglobin level 6.8 g/dL; range, 5.6-8.0 g/dL) and thrombocytopenia (median platelet count $85x10^9$/L; range, 67-$110x10^9$/L), with normal neutrophil count, except for patient #4 (neutrophil count $0.7x10^9$/L) and presented mild inflammation (median C-reactive protein 31 mg/L; range, 12.8-65 mg/L). Clinically, three were reported to have moderate obesity (patients #1, #2 and #3) and one had hepatosplenomegaly (patient #4). All had increased cortical density with diaphyseal involvement on bone X-rays, with a history of associated long-bone pain without skeletal deformity. Bone marrow aspirations failed, and biopsies revealed hypoplasia of the three myeloid lineages with dysmyelopoietic features, edema and fibrosis (*Online Supplementary Figure S1*). Investigations to rule out a myeloid malignancy were normal and genetic analyses for classical inherited bone marrow failure syndromes (Table 1) reported only the *TBXAS1* variants. The genetic diagnosis was made at a median time of 15.5 years (range, 4-34 years) after the first occurrence of symptomatic anemia.

Patients #3 and #4 had received high-dose steroids with a good response, but treatment was discontinued, due to relapse, after suspension and high-dose steroid requirement, respectively. Patient #1 had received hydrocortisone therapy at a dose of 100 mg/day with a weak response.

Before treatment with aspirin, patients #1, #2 and #4 required monthly RBC transfusions for over a year, while patient #3 was promptly treated with aspirin after the discontinuation of corticosteroids, due to worsening anemia, before requiring RBC transfusions. None of the patients required platelet transfusions.

The median age of the patients at the start of treatment with NSAID was 26 years (range, 23-40 years), corresponding to a median delay of 21 months (range, 1-48 months) from the first transfused anemia episode for patient #2, or from the relapse of anemia for the others. Aspirin was initiated at a dose of 75 mg/day, except for patient #3, who received 3 g/day (rheumatological dosage for rapid relief of bone pain).

All patients experienced a rapid hematologic response (Figure 1), with the hemoglobin level reaching >100 g/L without transfusion at a median of 43 days (range, 10-121 days), and were transfusion-independent at 1 month with resolution of bone pain. Their platelet counts exceeded $100x10^9$/L in less than 4 months. The neutrophil count of patient #4 improved to over $1.0x10^9$/L. The biological inflammatory syndrome initially resolved in all patients, concomitantly with improvement of the hematologic parameters.

During follow-up, patient #2 suddenly stopped the treatment for 2 months, resulting in a relapse of his anemia and inflammatory syndrome. A new complete hematologic response occurred 1 month after resumption of treatment, indicating an aspirin-dependence profile.

Patient #4 also relapsed on aspirin 75 mg/day, with the reappearance of an inflammatory syndrome followed by anemia. As suggested by Brown *et al.*,[4] patients may have different residual TBXAS1 enzyme activity, and in some cases require a higher dose of aspirin. Therefore, a dose of 500 mg/day was introduced; the current follow-up is too

Table 1. Patients' characteristics and main outcomes.

	Patient #1[a]	Patient #2[a]	Patient #3[a]	Patient #4[a]
Patients' characteristics				
Age in years at the start of NSAID	40	28	24	23
Aspirin dose	75 mg/day	75 mg/day	3 g/day	75 mg/day
Sex	Male	Male	Male	Male
Origin	Tunisian	Tunisian	Tunisian	Tunisian
Family history of GHD	No	No	One brother	No
Age at first disease manifestations	6 years	24 years	25 months	14 months
Spontaneous remission and duration	Yes 28 years	No	Yes 20 years	No
History of high-dose steroid treatment	No	No	Yes, steroid dependency	Yes, loss of efficacy
Age in years at molecular diagnosis	40	28	23	12
TBXAS1 mutations (bi-allelic)	c.1417G>T, p.Gly473Trp	c.1417G>T, p.Gly473Trp	c.245T>C, p.Leu82Pro	c.1417G>T, p.Gly473Trp
Specialized hematologic investigations				
Blood analysis				
Blood smear	No dysplasia	Rare dacrocytes	No dysplasia	No dysplasia
PNH clone	Negative	Negative	Negative	Small clone: 2%
Bone marrow aspiration				
Cytology examination	Diluted, non-contributory	Diluted, non-contributory	Diluted, non-contributory	Diluted, non-contributory
Karyotype	Failed	Normal (but limited to seven cells)	Normal	Normal
Chromosomal breakage analysis	Normal	Normal	Not performed	Normal
IBMF panel screening*	No additional mutation	No additional mutation	Not performed**	No additional mutation
Bone marrow biopsy				
Richness	Very severe hypoplasia	Severe hypoplasia, richness: 20%	Very severe hypoplasia, richness: grade 1	Severe hypoplasia, richness: grade 1-2
Medullary fibrosis***	Grade 1 with interstitial edema	Grade 1 with interstitial edema	Grade 3 + osteosclerosis	Grade 3
Affected hematologic lineages	Severe damage to all three lines	Severe damage to all three lines	Severe damage to all three lines	Severe damage to all three lines
Dysplasia	Signs of dysmega-karyopoiesis and dyserythropoiesis	Signs of dysmega-karyopoiesis and dyserythropoiesis	Signs of dysmega-karyopoiesis and dyserythropoiesis	No dysplastic features described
Clinical and biological parameters before treatment with NSAID				
Hemoglobin level, g/L	90	55	80	56
N of RBC units in the 6 months before starting NSAID treatment	14	6	0	12
Reticulocyte count, $x10^9$/L	N/A (5 months ago: 55.4)	50	N/A	55
Platelet count, $x10^9$/L	52	68	109	95
Neutrophil count, $x10^9$/L	1.8	2.3	2.4	0.7
CRP, mg/L	31	50	12.8	100
Radiological bone involvement[b]/ Bone pain	+/+	+/+	+/+	+/+

Continued on following page.

	Patient #1[a]	Patient #2[a]	Patient #3[a]	Patient #4[a]
Response to treatment				
Time in days to hemoglobin level response[c]	21	121	38	48
Time in days to platelet level response[d]	65	121	N/A	76
Persistent transfusion independency	Yes	Yes	Yes	No
Persistent hematologic response[e] at 6 months	Yes	Short relapse post-aspirin cessation, with a second complete response obtained 1 month after reintroduction	Yes	Loss of response with RBC transfusion required under treatment at 75 mg/day
Follow-up in months	17	14	52	9.5
Hematologic response at the end of follow-up	Yes	Yes	Yes	No

[a]No family relationship between the patients could be established on examination. [b]Bone abnormality was defined as long-bone X-rays revealing an increased cortical density with diaphyseal involvement, often associated with bone pain. [c]Hemoglobin level response is defined as a hemoglobin level ≥100 g/L without transfusion in the preceding month. [d]Platelet level response is defined as a platelet level ≥100x10^9/L without transfusion in the preceding month. [e]Hematologic response is defined as an association of hemoglobin level and platelet level responses, with no bone pain related to the disease. It is counted as persistent if no relapse episode has been recorded since it was obtained. *The screening panel for innate bone marrow failure (IBMF) syndromes includes *ARID2, ASXL1, ASXL2, ATRX, BCOR, BCORL1, BRAF, BRCA1, BRCA2, BRCC3, CALR, CBL, CEBPA, CHEK2, CLPB, CREBBP, CSF3R, CSNK1A1, CTCF, CUX1, DDX41, DNMT3A, ELANE, EP300, ERCC6L2, ETNK1, ETV6, EZH2, FLT3, GATA2, HRAS, IDH1, IDH2, IKZF5, IRF1, JAK2, JAK3, KDM5A, KDM6A, KIT, KMT2A/MLL, KMT2D/MLL2, KRAS, LUC7L2, MBD4, MECOM, MPL, MPO, MYC, NF1, NPM1, NRAS, PDS5B, PHF6, PPM1D, PRPF8, PTEN, PTPN11, RAD21, RIT1, RUNX1, SAMD9, SAMD9L, SBDS, SETBP1, SF1, SF3B1, SMC1A, SMC3, SRP72, SRSF2, STAG2, TET2, TERC, TERT, TP53, U2AF1, U2AF2, WT1, ZNF687, ZRSR2,* and *TBX1S1. TBXAS1* is currently included in the IBMF panel, but was also studied specifically in our patients. **For patient #3, given that his brother had confirmed Ghosal hematodiaphyseal dysplasia, a targeted investigation was carried out with confirmation of the *TBXAS1* mutation and the use of a restricted panel to screen for myeloid disorders. *** The bone marrow biopsies from patients #1 and #2 were reassessed by two specialized hematopathologists who reported that the fibrosis observed is unusual with, notably, interstitial edema. NSAID: non-steroidal anti-inflammatory drugs; GHD: Ghosal hematodiaphyseal dysplasia; PNH: paroxysmal nocturnal hemoglobinuria; IBMF: innate bone marrow failure; N of RBC units: number of red blood cell units; N/A: not available; CRP: C-reactive protein.

short to assess response. Patient #4 was the only patient with a chronic disease evolution since childhood, associated with the emergence of steroid resistance and clinical hepatosplenomegaly, probably related to extensive bone marrow fibrosis. Another consideration, if improvement stalls despite dose escalation, is that the prolonged illness may have severely affected the bone marrow, hindering effective hematopoiesis through treatment.

With a median follow-up of 15.5 months (range, 9.5-52 months), patients #1, #2 and #3 sustained a complete hematologic response (on aspirin 75 mg/day for patients #1 and #2, and on a reduced dose of 2 g/month for patient #3, due to persistent remission).

Considering the spontaneous hematologic improvement that occurred during childhood in patients #1 and #3, we looked for a correlation between the phenotype and *TBXAS1* genotype. Analysis of our patients and genetically confirmed cases in the literature[3-10] (*Online Supplementary Table S1*) revealed three phenotypes, summarized in Table 2.

Firstly, there was a group of hematologically asymptomatic carriers, or with at worse moderate anemia, with no reported history of RBC transfusions (group 1, n=5). These subjects were diagnosed at a median age of 16 years, during family screening performed because of a symptomatic relative. Although they all had abnormal bone X-rays consistent with GHD, no bone symptoms were reported. Interestingly, most were female (80%).

The second phenotypic group (group 2, n=4) comprised patients with childhood onset of severe anemia, necessitating RBC transfusions (discovered at a median of 25 months), who achieved a spontaneous hematologic improvement after a mean time of 3.5 years (range, 1-6.5), allowing RBC transfusions to be stopped. Three patients relapsed after a prolonged hematologically asymptomatic period (12.5 to 28 years), presenting with severe transfused anemia with clinical and radiological bone manifestations and a biological inflammatory syndrome.

The last group (group 3, n=14) included patients with early severe and persistent/long-lasting transfusion-dependent anemia (median age at onset of 2 years), associated with thrombocytopenia and leukopenia in most cases. They all had radiological bone involvement, with half of them experiencing bone pain. Twelve patients were treated with high-dose corticosteroids as first-line therapy (the other 2 received NSAID as first-line treatment), with a good

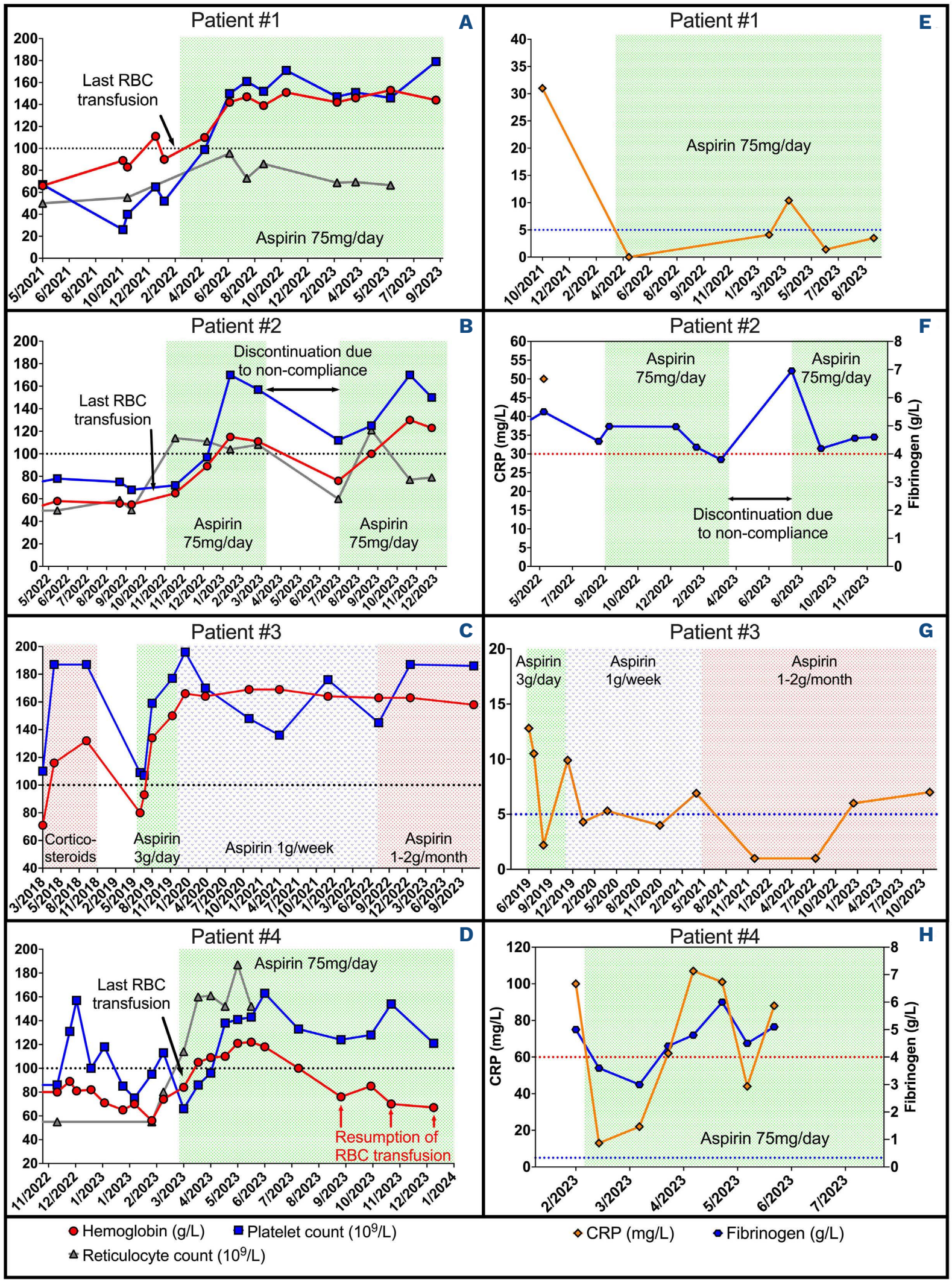

Figure 1. The main hematologic and inflammatory biological parameters during treatment of the four patients with Ghosal hematodiaphyseal dysplasia. (A-D) Hematological results. (E-H) Inflammatory results. The standards for a positive determination were set at >5 mg/L for C-reactive protein, represented by a dashed red line, and for fibrinogen at >4g/L, represented by the dashed blue line. The shadowed background represents the evolution of results under aspirin treatment. The green color indicates the initial aspirin dose, while the change in color corresponds to a change in dose. For patient #4, inflammatory results were missing at relapse and at the end of follow-up. RBC: red blood cell; CRP: C-reactive protein.

hematologic response in each. However, only 25% of these patients were able to stop steroids without relapse (data available for 8 patients with a short follow-up).

No genotype/phenotype correlations were identified (*Online Supplementary Table S2*). Inter- and intra-familial heterogeneity suggest unknown factors affecting phenotype, such as, possibly, female sex, which was more frequent in asymptomatic patients (group 1: 80% *vs*. groups 2+3: 20%). In groups 2 and 3 the appearance of anemia coincided with a biological inflammatory syndrome, which may reflect disease activity. External factors, such as infections, might also trigger disease worsening by generating inflammation and stimulating the arachidonic acid pathway, exacerbating metabolic problems caused by *TBXAS1* mutations, and contributing to clinical heterogeneity. However, grouping patients is challenging, due to the heterogeneity of the population and the reported data, but also due to the lack of information on follow-up for asymptomatic patients and the impact of treatment on spontaneous outcome of the disease.

Our four GHD patients, treated with low-dose aspirin, confirm this treatment's efficacy with three sustained hematologic responses. Our last patient, despite an initial response, relapsed and is currently being treated with a higher dose. Hypotheses to explain the loss of response were non-compliance, drug interaction and a more severe enzymatic defect. Considering the treatment's excellent tolerability, especially its lower infection risk compared with corticosteroids, low-dose NSAID appear promising. However, data on the efficacy of NSAID in correcting radiological bone lesions are lacking, unlike steroid treatment, for which some pediatric studies have reported improvements.

Our review of the literature highlights the clinical heterogeneity of GHD, including the possibility of long-lasting spontaneous hematologic improvement in childhood, which has been rarely reported so far. In most of these patients, anemia required RBC transfusions over a prolonged period, supporting treatment with low-dose NSAID as soon as transfusions are required. No genotypic or clinical predictive factors were identified to predict a spontaneous improvement, which would have helped us to discuss the discontinuation of treatment in some patients. For others, such as patient #2, who relapsed quickly after treatment was discontinued, suggesting NSAID dependency, lifelong treatment would seem necessary.

Lastly, our study highlights the importance of considering GHD in adults, as well as in children presenting with severe anemia and thrombocytopenia, especially in the case of abnormally tough bones, independently of myelofibrosis or radiological findings. Given the low toxicity of aspirin, *TBXAS1* mutation should be added to the panel for inherited bone marrow failure screening, especially if transplantation

Table 2. Subgroup phenotype description.

	All	Group 1: Asymptomatic without history of RBC transfusion	Group 2: Spontaneous hematologic remission history Data at first episode (data at relapse)	Group 3: Symptomatic without history of spontaneous remission
N	23	5	4 (relapse: 3)	14
Age, median (range)*	6 yrs (5 mths-34 yrs)	16 yrs (6-24 yrs)	4 yrs (18 mths-6 yrs)	2 yrs (5 mths-34 yrs)
Sex	16 M/7 F	1 M/4 F	3 M/1 F (2 M/1 F)	12 M/2 F
Bone pain	4/10	0/2	1/1 (3/3**)	4/8
Pathological bone X-ray	23/23	5/5	2/2 (2/3)***	14/14
Anemia	20/23	2/5 (moderate)	4/4 (3/3)	14/14
RBC transfusion	13/19	0/5	4/4 (3/3)	8/9
Thrombopenia	15/22	0/5	3/4 (3/3)	12/13
Leukopenia	6/20	0/5	0/2 (0/3)	5/13
Inflammatory syndrome	10/17	0/5	0/1 (3/3)	10/14

Asymptomatic means no hematologic disorders. *Age at first symptoms or at diagnosis if asymptomatic. **Not reported for three patients at first symptoms but all of them had bone pain at relapse. ***Two patients had pathological bone radiography at diagnosis, the two other patients had pathological bone radiography at relapse (no data for first episode), all patients have a history of pathological bone radiography typical of Ghosal hematodiaphyseal dysplasia during their lifetime. RBC: red blood cell; N: number of patients; yrs: years; mths: months; M: male; F: female.

is considered. Unlike other inherited bone marrow failure syndromes, no evolution to myeloid malignancy has been reported. While most reported cases involved children without long-term follow-up, five patients (four from our study) provided reassuring follow-up data into their fourth decade.

Authors

Jonathan Bordat,[1] Felipe Suarez,[2-4] Valérie Cormier-Daire,[2,5] Regis Peffault de Latour,[1,2,6] Jean Soulier,[2,6,7] Veronique Meignin,[8] Mathilde Doyard,[5] Lise Larcher,[2,6,7] Stephane Vanderbecken[9] and Flore Sicre de Fontbrune[1,6]

[1]Department of Hematology and Bone Marrow Transplantation, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris, Paris; [2]Paris Cité University, Paris; [3]Imagine Institute, INSERM UMR 1163, Laboratory of Molecular Mechanisms of Hematologic Disorders and Therapeutic Implications, Necker-Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris; [4]Adult Hematology, Necker-Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris; [5]French Reference Center for Skeletal Dysplasia, INSERM UMR 1163, Imagine Institute, Necker Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris, Paris; [6]French Reference Center for Aplastic Anemia, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris, Paris; [7]Laboratory of Hematology, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris, Paris; [8]Department of Pathology, Saint-Louis Hospital, Assistance Publique-Hôpitaux de Paris, Paris and [9]Hemophilia Treatment Center, University Hospital of Réunion, Réunion Island, France.

Correspondence:
J. BORDAT - jonathan.bordat@aphp.fr
F. S. DE FONTBRUNE - flore.sicre-de-fontbrune@aphp.fr

https://doi.org/10.3324/haematol.2023.284098

Received: October 27, 2023.
Accepted: February 28, 2024.
Early view: March 14, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

JB and FSdF designed the study, collected the patients and wrote the original version of the manuscript. FSdF, FS, RPdL, and SV took care of the patients and enrolled them in the RIME database. VCD, JS, MD, and LL were responsible for molecular data analysis and performed the genetic diagnosis. VM provided data about bone marrow biopsies and pictures for *Online Supplementary Figure S1*. All authors contributed to reviewing and editing the manuscript and approved the final version.

Data-sharing statement

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

References

1. Ghosal SP. An unusual bone dysplasia. Indian J Pediatr. 1957;24(12):359-368.
2. Isidor B, Dagoneau N, Huber C, et al. A gene responsible for Ghosal hemato-diaphyseal dysplasia maps to chromosome 7q33-34. Hum Genet. 2007;121(2):269-273.
3. Geneviève D, Proulle V, Isidor B, et al. Thromboxane synthase mutations in an increased bone density disorder (Ghosal syndrome). Nat Genet. 2008;40(3):284-286.
4. Brown TJ, Barrett NA, Meng H, et al. Non-steroidal anti-inflammatory drugs as a targeted therapy for bone marrow failure in Ghosal hematodiaphyseal dysplasia. Blood. 2023;141(13):1553-1559.
5. Jeevan A, Doyard M, Kabra M, Daire VC, Gupta N. Ghosal type hematodiaphyseal dysplasia. Indian Pediatr. 2016;53(4):347-348.
6. Joy P, Yoganathan S, Korula S, et al. Ghosal hematodiaphyseal dysplasia and response to corticosteroid therapy. Am J Med Genet A. 2021;185(2):596-599.
7. Kim SY, Ing A, Gong S, Yap KL, Bhat R. Novel compound heterozygous variants of TBXAS1 presenting with Ghosal hematodiaphyseal dysplasia treated with steroids. Mol Genet Genomic Med. 2021;9(3):e1494.
8. Sharma R, Sierra Potchanant E, Schwartz JE, Nalepa G. Chronic steroid-response pancytopenia and increased bone density due to thromboxane synthase deficiency. Pediatr Blood Cancer. 2018;65(1):e26777.
9. Sudhakar M, Sharma M, Kandasamy S, Gummadi A, Rawat A, Vignesh P. Novel TBXAS1 variants in two Indian children with Ghosal hematodiaphyseal dysplasia: a concise report. Eur J Med Genet. 2022;65(5):104498.
10. Selina A, Kandagaddala M, Madhuri V. A recurrent biallelic pathogenic variant in TBXAS1 gene causing Ghosal hematodiaphyseal dysplasia. Indian J Pediatr. 2021;88(4):381-382.