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**Hydroxyurea Therapy in UK Children with Sickle Cell Anaemia – A Single Centre Experience**Kate Phillips PhD<sup>1</sup>, Laura Healy DipHE<sup>2</sup>, Louise Smith RCN MSc<sup>2</sup> & Russell Keenan MBChB, PhD, FRCP, FRCPath<sup>2</sup><sup>1</sup>School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF<sup>2</sup>Department of Haematology, Alder Hey Children's NHS Foundation Trust, Eaton Road, Liverpool, L12 2AP

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**Abbreviations**

FBC	Full blood count
Hb	Haemoglobin
HbF	Foetal haemoglobin
MTD	Maximum tolerated dose
SCA	Sickle cell anaemia
TCD	Transcranial Doppler
VDJ	Variable diversity joining

**Abstract:****Introduction**

Despite the demonstrated efficacy of hydroxyurea therapy, children with sickle cell anaemia in the United Kingdom (UK) are preferentially managed with supportive care or transfusion. Hydroxyurea is reserved for children with severe disease phenotype. This is in contrast to North America and other countries where hydroxyurea is widely used for children of all clinical phenotypes. The conservative UK practice may in part be due to concerns about toxicity, in particular marrow suppression with high doses, and growth in children.

**Methods and Results**

We monitored 37 paediatric patients with sickle cell anaemia who were treated with hydroxyurea at a single UK treatment centre. Therapy was well tolerated and mild transient cytopenias were the only toxicity observed. Comparative analysis of patients receiving  $\geq 26$  mg/kg/day versus  $< 26$  mg/kg/day demonstrates increasing dose has a significant positive effect on foetal haemoglobin (29.2% v 20.4%,  $p=0.0151$ ), mean cell volume (94.4 v 86.5,  $p=0.0183$ ) and reticulocyte count ( $99.66 \times 10^9/L$  v  $164.3 \times 10^9/L$ ,  $p=0.0059$ ). Marrow suppression was not a clinical problem with high dose treatment, haemoglobin 92.25 g/L v 91.81 g/L (ns), neutrophil count  $3.3 \times 10^9/L$  v  $4.8 \times 10^9/L$  (ns) and platelet count  $232.4 \times 10^9/L$  v  $302.2 \times 10^9/L$  (ns). Normal growth rates were maintained in all children. Good adherence to therapy was a significant factor in reducing hospitalisations

**Conclusion**

This study demonstrates the effectiveness and safety in practice of high dose hydroxyurea as a disease modifying therapy which we advocate for all children with sickle cell anaemia.

## 1 **Introduction**

2 In children with sickle cell anaemia (SCA), hydroxyurea therapy is effective at elevating foetal  
3 haemoglobin (HbF) and reducing the frequency of painful sickle crises[1-3]. Despite the demonstrated  
4 efficacy of hydroxyurea therapy, children with SCA are preferentially managed with supportive care or  
5 transfusion at United Kingdom (UK) treatment centres. Current UK clinical guidance is supportive  
6 care and prophylactic antibiotics for all children, and transfusion in instances of acute and chronic  
7 complications such as acute anaemia, acute chest syndrome, acute neurological deficit, stroke  
8 prevention and organ failure. Hydroxyurea is restricted to those with severe phenotype. Current  
9 guidelines recommend it is considered only in patients who experience recurrent episodes of acute pain  
10 (more than 3 hospitalisations in the previous 12 months or symptomatic in the community) or who have  
11 experienced two or more episodes of acute chest syndrome[4]. Referral to a specialist centre is required  
12 for hydroxyurea commencement and it is recommended that the patient and parents or carers have two  
13 separate documented discussions with treating clinicians, to include side effects such as subfertility,  
14 cytopenias and the possible risk of leukaemia or other malignancies[4]. The effect of such guidance is  
15 that few children with SCA in the UK benefit from hydroxyurea therapy. Currently 1.9% of UK patients  
16 with SCA <5 years of age receive hydroxyurea, and 11.3% of 5-17 year old UK SCA patients receive  
17 hydroxyurea[5].

18 Reluctance to incorporate hydroxyurea into first line treatment in the UK relates in part to concerns  
19 over long term safety and negative effects on growth, combined with a lack of awareness of potential  
20 treatment benefits. Since publication of the current UK Standards and Guidance for Clinical Care  
21 (2010), much more evidence as to the safety and efficacy of hydroxyurea therapy has become  
22 available[2,6-10]. In the USA, it is now recommended that all children with sickle cell anaemia be  
23 offered hydroxyurea therapy from 9 months of age[11].

24 Concerns over potential negative effects on growth were addressed initially in the 1999 HUG-KIDS  
25 phase I/II trial. Fifty two severely affected children aged 5-15 years were treated to maximum tolerated  
26 dose (MTD) with a median dose of 30mg/kg/day hydroxyurea for 1 year and no child experienced  
27 growth failure[12]. Following on from this, the HUSOFT extension study examined the effects of

28 longer term treatment (up to 6 years) on 21 very young children, and reported that females maintained  
29 average growth rates and males increased average growth rates during hydroxyurea therapy[13].

30 Since hydroxyurea inactivates the enzyme ribonucleoside diphosphate reductase[14], it impairs both  
31 DNA replication and repair. This gives rise to concerns as to its carcinogenic and teratogenic potential.  
32 So far, two follow up studies have investigated the risks and benefits of very long-term use in adults  
33 [15,16]. The Multicentre Study of Hydroxyurea in Sickle Cell Anaemia trial had a 17.5 year follow up  
34 and some participants had received greater than 15 years cumulative exposure. This study found there  
35 was no increase in neoplasia by exposure to hydroxyurea[15]. Another single centre trial, where 131  
36 adults were followed for up to 17 years, also reported that no cases of carcinogenesis occurred[16].  
37 Importantly, both of these studies reported significantly improved survival in hydroxyurea treated  
38 patients[15,16]. More recently, investigators in the BABY-HUG phase III clinical trial sought to  
39 measure acquired genomic damage in infants, as a measure of carcinogenic potential. Karyotype  
40 analysis (to investigate chromosome/chromatid breaks), illegitimate variable-diversity-joining (VDJ)  
41 recombination events and micronucleated reticulocytes were monitored and there were no differences  
42 between hydroxyurea and placebo treated patients[10].

43 Since the reporting of the BABY-HUG trial results, we have offered hydroxyurea as a first line  
44 treatment to all children over 9 months of age with SCA at this UK centre. Here, we sought to review  
45 the benefits of hydroxyurea therapy in a UK paediatric cohort, and to determine the effect of dose  
46 escalation and adherence to therapy in terms of improvement in haematological parameters and hospital  
47 admissions.

#### 48 **Methods**

49 All children over 9 months of age were offered hydroxyurea therapy and no selection for disease  
50 severity was made. Baseline investigations were performed to confirm the absence of anaemia  
51 (haemoglobin (Hb)  $>50\text{g/L}$ ), absence of cytopenias (neutrophils  $>1.0 \times 10^9/\text{L}$ , platelets  $>100 \times 10^9/\text{L}$ ,  
52 reticulocytes  $>40 \times 10^9/\text{L}$ ) and absence of severe hepatic or renal impairment (estimated glomerular  
53 filtration rate  $>30\text{mL}/\text{min}/1.73\text{m}^2$ ). Patients were not considered for hydroxyurea therapy if they had

54 known hypersensitivity to hydroxyurea, were pregnant, had active hepatitis, showed any signs of  
55 myelosuppression or were receiving anti-retroviral therapy.

56 Treatment commenced at 15 mg/kg/day to be increased over the following 12 months to MTD or 35  
57 mg/kg/day, whichever was achieved first. MTD was defined by haematological toxicity as the  
58 maximum dose where no myelosuppression was induced. On commencement of therapy, full blood  
59 counts (FBC) were monitored fortnightly for the first 4 weeks and then at 4 weekly intervals. Clinical  
60 assessment was made monthly and included frequency and severity of crises, complications of sickle  
61 cell disorders, compliance with treatment, adverse events, height and weight measurements and  
62 laboratory monitoring of hepatic and renal function, reticulocytes, FBC and haemoglobin  
63 electrophoresis. Dose was increased by 5mg/kg/day every 8 weeks, provided that no marrow toxicity  
64 was observed (defined as neutrophil count  $<0.75 \times 10^9/L$  or platelet count  $<75 \times 10^9/L$  or reticulocyte  
65 count  $<40 \times 10^9/L$ ). FBCs were monitored fortnightly following dose increases for 4 weeks, and then  
66 at 4 weekly intervals.

## 67 **Results**

68 Ninety five percent of children and parents or carers offered hydroxyurea consented following  
69 discussion of current evidence in relation to side effects and benefits of treatment ( $n = 37$ ). Hydrea in  
70 500mg capsule or liquid formulation was prescribed. The mean age at start of therapy in our patients  
71 was 7 years (range 1 – 15), 34 patients were HbSS, 2 were HbS $\beta^0$  thalassemia and 1 was HbSD. The  
72 mean dose achieved in patients who had been on therapy for more than 6 months was 28.5 mg/kg/day.

73 Significant improvements were recorded in haematological parameters post-hydroxyurea therapy; Hb  
74 increased to 92.0 g/L from 80.6 g/L, HbF increased to 26.35% from 9.90%, MCV increased to 91.06  
75 from 77.49, reticulocytes decreased to  $127.6 \times 10^9/L$  from  $335.4 \times 10^9/L$  and neutrophils decreased to  
76  $3.95 \times 10^9/L$  from  $6.37 \times 10^9/L$  ( $n=37$ ;  $p<0.001$  by paired t-test for each parameter; supplemental figure  
77 1 (S1)). A significant reduction in platelets was also recorded in our patients (to  $263.3 \times 10^9/L$  from  
78  $352.4 \times 10^9/L$ ;  $p=0.0007$ ; supplemental figure 1 (S1)).

79 At the time of review, 16 patients were receiving  $<26$  mg/kg/day hydroxyurea and 21 patients were  
80 receiving  $\geq 26$  mg/kg/day. Comparative analysis of patients receiving  $<26$  and  $\geq 26$  mg/kg/day indicated  
81 that dose had a significant effect on some parameters. Hb levels were increased post-hydroxyurea  
82 therapy irrespective of dose achieved (Figure 1A). Whereas HbF level, MCV and reticulocyte count  
83 were further improved in patients receiving  $\geq 26$  mg/kg/day (Figure 1B, 1C & 1D). Neutrophil and  
84 platelet counts were only significantly reduced in patients receiving  $\geq 26$ mg/kg/day (Figure 1E & 1F).  
85 Patient age versus dose achieved is shown in Figure 1G.

86 Haemolysis was better controlled in patients receiving  $\geq 26$ mg/kg/day. Bilirubin and lactate  
87 dehydrogenase (LDH) were significantly lower in patients receiving  $\geq 26$  mg/kg/day (Figure 2A & 2B).  
88 Concurrent monitoring of alanine amino transferase (ALT) and aspartate amino transferase (AST)  
89 showed no evidence of liver toxicity in any patient, although AST was significantly reduced in patients  
90 receiving  $\geq 26$ mg/kg/day (Figure 2C & 2D). Serum creatinine and urea were monitored continuously  
91 and remained within age appropriate reference range for all patients.

92 Adherence to therapy was reported by the patient and parent or carer at each clinic visit. We identified  
93 10 patients with poor adherence to therapy based on patient/parent/carer admission and confirmed that  
94 they had not collected prescriptions regularly enough to maintain prescribed daily dosing. Comparative  
95 analysis between these patients with poor adherence, and all other patients who were considered to have  
96 good adherence (based on patient/parent/carer reporting), indicates that improvement in haematological  
97 parameters is dependent on good adherence to therapy (Figure 3). Hb and HbF levels were significantly  
98 higher in patients with good adherence (Figure 3A & 3B). As would be expected, MCV was  
99 significantly higher with good adherence (Figure 3C), and reticulocyte and neutrophil counts were  
100 significantly lower (Figure 3D & 3E). Adherence had no significant effect on platelet count (Figure  
101 3F) or haemolysis (Bilirubin/LDH; Figure 3G & 3H) however, both ALT and AST were significantly  
102 reduced with good adherence which may indicate beneficial effects on hepatic function (Figure 3I and  
103 3J).

104 Adherence to therapy also had a significant impact on the number of hospitalisations experienced by  
105 patients. Twenty seven hospitalisations totalling 126 admission days were recorded for the 10 patients  
106 with poor adherence, compared to 9 hospitalisations totalling 49 admission days in the 27 patients with  
107 good adherence ( $p < 0.0001$  by  $\chi^2$  test; 24 month follow up). Height and weight were monitored  
108 continuously for all patients and growth rates were maintained in all children irrespective of dose  
109 achieved or adherence to therapy (Figure 4).

## 110 **Discussion**

111 Our data demonstrates the significant improvements in haematological parameters that can be achieved  
112 with hydroxyurea therapy in UK paediatric patients with SCA. All children in this study had previously  
113 been managed at UK treatment centres with supportive care or transfusions. Importantly, hydroxyurea  
114 resulted in significant improvements in Hb, HbF, MCV, reticulocyte and neutrophil count which were  
115 apparent within 6 months of therapy commencement. Our data indicates that dose escalation is required  
116 to maximise beneficial effects, particularly in respect of HbF where patients receiving  $\geq 26\text{mg/kg/day}$   
117 achieved a median HbF level of 33.80%. HbF may protect HbS from de-oxygenation and  
118 polymerisation through local oxygen buffering, and is considered a good indicator of clinical disease  
119 severity[17]. Escalating dose to  $\geq 26\text{mg/kg/day}$  exerted better control of haemolysis with increased Hb  
120 and decreased bilirubin, LDH and reticulocytes. Our study also highlights the critical importance of  
121 good adherence to therapy, particularly in respect to hospitalisations and admission days.

122 An increasing body of literature exists demonstrating the efficacy of hydroxyurea in SCA. The most  
123 notable recent demonstration in paediatric patients is arguably the BABY-HUG multicentre randomised  
124 controlled trial[2]. Similar to the improvements seen here, the BABY-HUG investigators reported  
125 significant improvements in Hb, HbF, MCV, reticulocyte count and neutrophil count in hydroxyurea  
126 treated patients. Further, the BABY-HUG investigators reported improvements in renal function and  
127 cerebral artery blood flow based on transcranial Doppler (TCD) velocity. Clinical events were also  
128 improved, with significantly reduced incidences of pain, acute chest syndrome, hospitalisation,  
129 transfusion, dactylitis and gastroenteritis[2].



130 The UK paediatric patient cohort in this study benefited from hydroxyurea therapy as expected based  
131 on previous reports of hydroxyurea use in SCA[2,12,13,18]. As such we believe it is now time to  
132 address the barriers to therapy that exist within the UK. No negative effects on growth were observed  
133 in this study and the only treatment related toxicities encountered were cytopenias, most frequently mild  
134 cytopenias which resolved without the need for dose reduction.

135 We considered neutrophil count  $<0.75 \times 10^9/L$ , or platelet count  $<75 \times 10^9/L$ , or reticulocyte count  $<40$   
136  $\times 10^9/L$  clinically significant toxicities. Nine of the 37 children treated experienced significant  
137 cytopenia, totalling 22 separate episodes. Three children experienced a single episode (2  
138 reticulocytopenia, 1 thrombocytopenia) while being clinically well. Laboratory monitoring was  
139 increased to weekly and counts recovered within 2 weeks (thrombocytopenia) and 6 weeks  
140 (reticulocytopenia) without the need for dose adjustment. One child experienced two episodes of  
141 reticulocytopenia 8 months apart while being clinically well, and 1 child experienced two episodes of  
142 thrombocytopenia 15 months apart while being clinically well. Both children were managed with  
143 increased laboratory monitoring and counts recovered within 2 weeks (thrombocytopenia) or 3 weeks  
144 (reticulocytopenia) without the need for dose adjustment. Three children experienced cytopenias  
145 following dose increase or alongside falling haemoglobin or neutrophil count, which required dose  
146 adjustment (6 thrombocytopenia and 1 reticulocytopenia). Hydroxyurea therapy was stopped and  
147 laboratory monitoring increased to weekly. Hydroxyurea was re-commenced following recovery of  
148 counts, which occurred within 1 – 3 weeks. Therapy was recommenced at the same dose for children  
149 receiving  $<20\text{mg/kg/day}$  or at a reduced dose for children receiving  $>20\text{mg/kg/day}$ . One child with  
150 severe phenotype experienced 7 episodes of cytopenia over 21 months (4 neutropenia, 3  
151 reticulocytopenia). Each cytopenia was managed with increased laboratory monitoring and resolved  
152 without the need for dose reduction. We persisted with hydroxyurea therapy for this patient as  
153 significant improvement in clinical features were seen, including reduced crisis, return of conditional  
154 TCD to normal and increase in HbF from 7.0% to 32.7%. In our experience, isolated cytopenias are  
155 best managed initially with increased laboratory monitoring (weekly) while maintaining dose in

156 clinically well children. Dose reduction or therapy cessation should be considered when an isolated  
157 cytopenia is persistent or deteriorates, or if more than one haematological parameter is affected.

158 While hydroxyurea therapy resulted in clinical improvement, in comparison to a supportive care  
159 approach, it also resulted in increased medication related burden on patients and families. In this  
160 respect, when children are established on a stable dose it may be possible to reduce monitoring to  
161 bimonthly to counteract some of this additional burden[20]. Achieving accurate dosing in children  
162 prescribed 500mg capsules frequently required an 'asymmetric' daily dosing schedule to achieve a  
163 desired weekly intake, and this adds an additional layer of complexity to daily administration. However,  
164 these issues were overcome in our patients and their families who were motivated by tangible treatment  
165 benefits such as improvements in laboratory values.

166 Currently, the UK National Haemoglobinopathy Registry shows only 9.5% of all UK patients with SCA  
167 <18 years of age receive hydroxyurea therapy[5]. In the absence of substantive evidence to contradict  
168 safety, we advocate the use of hydroxyurea as a disease modifying therapy for all UK children with  
169 SCA. Future UK clinical guidelines should place more focus on achievable benefits with hydroxyurea,  
170 and broaden the application to many more children.

#### 171 **Conflict of interest**

172 The authors declare they have no competing interests.

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### 235 **Legends**

236 **Figure 1. Hydroxyurea (HU) improves haematological parameters.** Increased Hb (A), increased  
 237 HbF (B), increased MCV (C) and decreased reticulocytes (D) were observed post-treatment irrespective  
 238 of dose achieved, although treating with  $\geq 26$ mg/kg further improved HbF, MCV and reticulocyte count.  
 239 Treatment with  $\geq 26$ mg/kg also resulted in decreased neutrophils (E) and platelets (F). Lines indicate  
 240 mean  $\pm$  SD, statistics by paired t-test for pre/post treatment comparison and by t-test for dose  
 241 comparison. Dose achieved did not correlate with patient age (G), dotted line indicates 26mg/kg  
 242 threshold used for dose comparison.

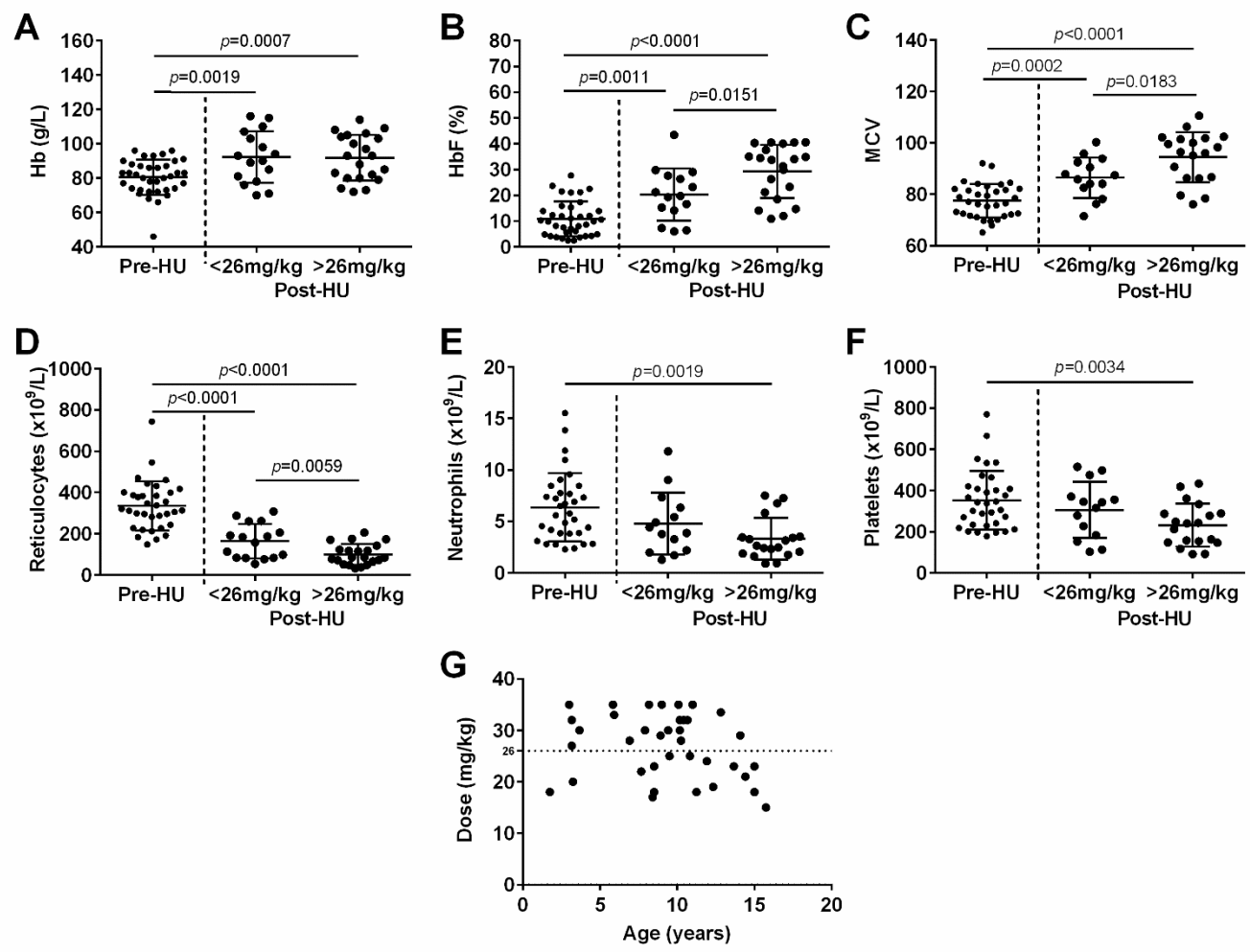
243 **Figure 2. Hydroxyurea (HU) improves control of haemolysis.** Bilirubin (A) and lactate  
 244 dehydrogenase (LDH; B) were reduced in children receiving  $\geq 26$ mg/kg hydroxyurea. Alanine amino  
 245 transferase (ALT) remained stable irrespective of dose (C) and aspartate amino transferase (AST) was  
 246 reduced in children receiving  $\geq 26$ mg/kg (D). Lines indicate mean  $\pm$  SD, statistics by paired t-test for  
 247 pre/post treatment comparison and by t-test for dose comparison. Grey shading indicates normal  
 248 reference range for each parameter measured.

249 **Figure 3. Effects of good adherence to therapy.** Good adherence to therapy had a significant effect  
 250 on Hb (A), HbF (B), MCV (C), reticulocyte count (D) and neutrophil count (E) but not on platelet count  
 251 (F). No significant effects were recorded in bilirubin (G) or LDH (H) but ALT (I) and AST (J) were

252 significantly reduced by good adherence to therapy. Lines indicate mean  $\pm$  SD, statistics by t-test, grey  
253 shading represents normal reference range for each parameter (G-J).

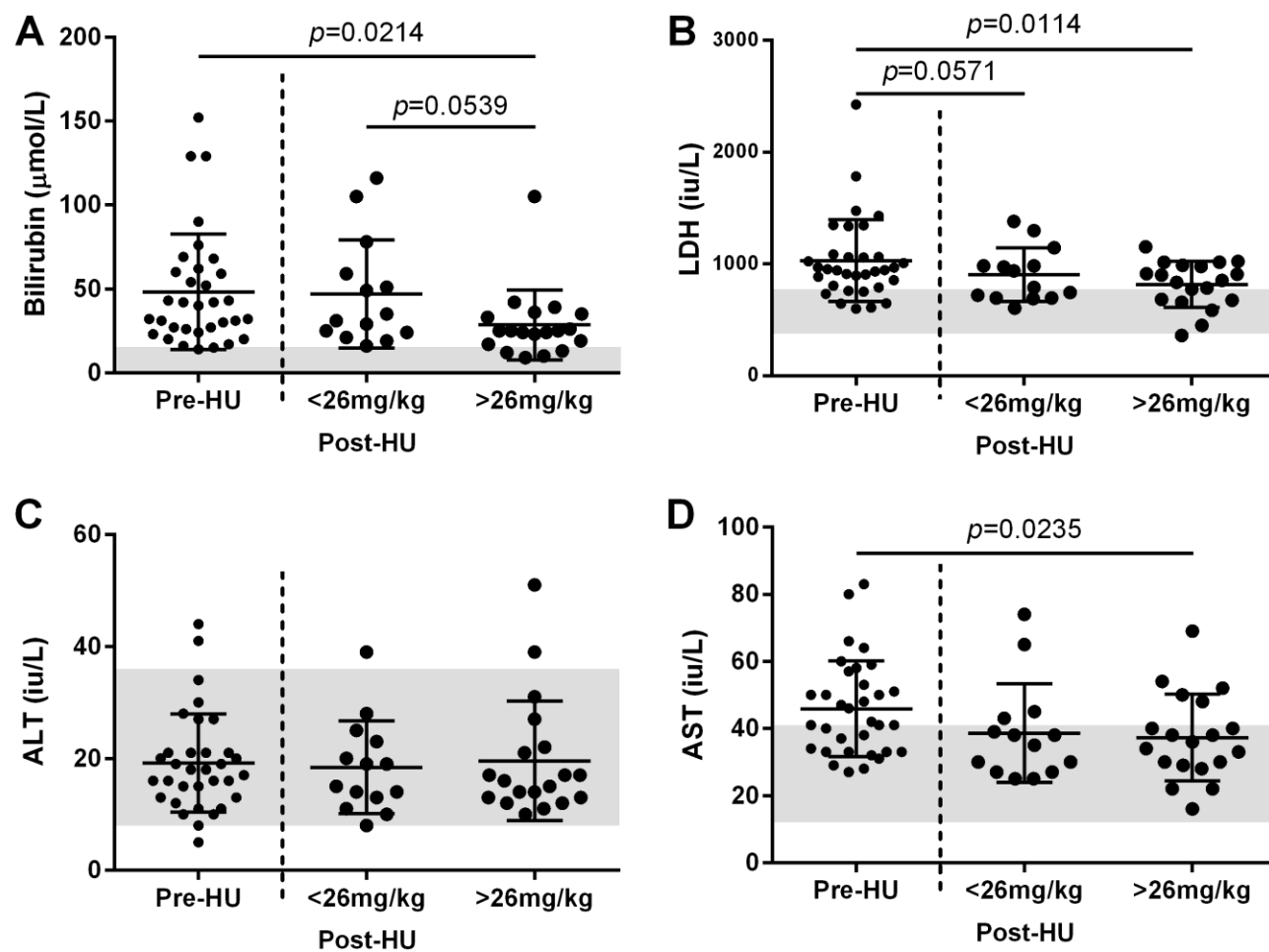
254 **Figure 4. Growth rates in children following hydroxyurea therapy.** Height (A) and weight (B)  
255 were maintained in children treated with hydroxyurea. Arrow indicates commencement of hydroxyurea  
256 therapy. Data shown is height for age (A) and weight for age (B) z-score calculated using CDC growth  
257 chart[19]

258 **Supplemental Figure 1. Hydroxyurea (HU) improves haematological parameters.** Significant  
259 improvements in haemoglobin (Hb), foetal haemoglobin (HbF), mean cell volume (MCV), reticulocyte  
260 count and neutrophil count were recorded post-HU therapy in UK paediatric patients with sickle cell  
261 anaemia. Platelets were also significantly reduced post-HU therapy.



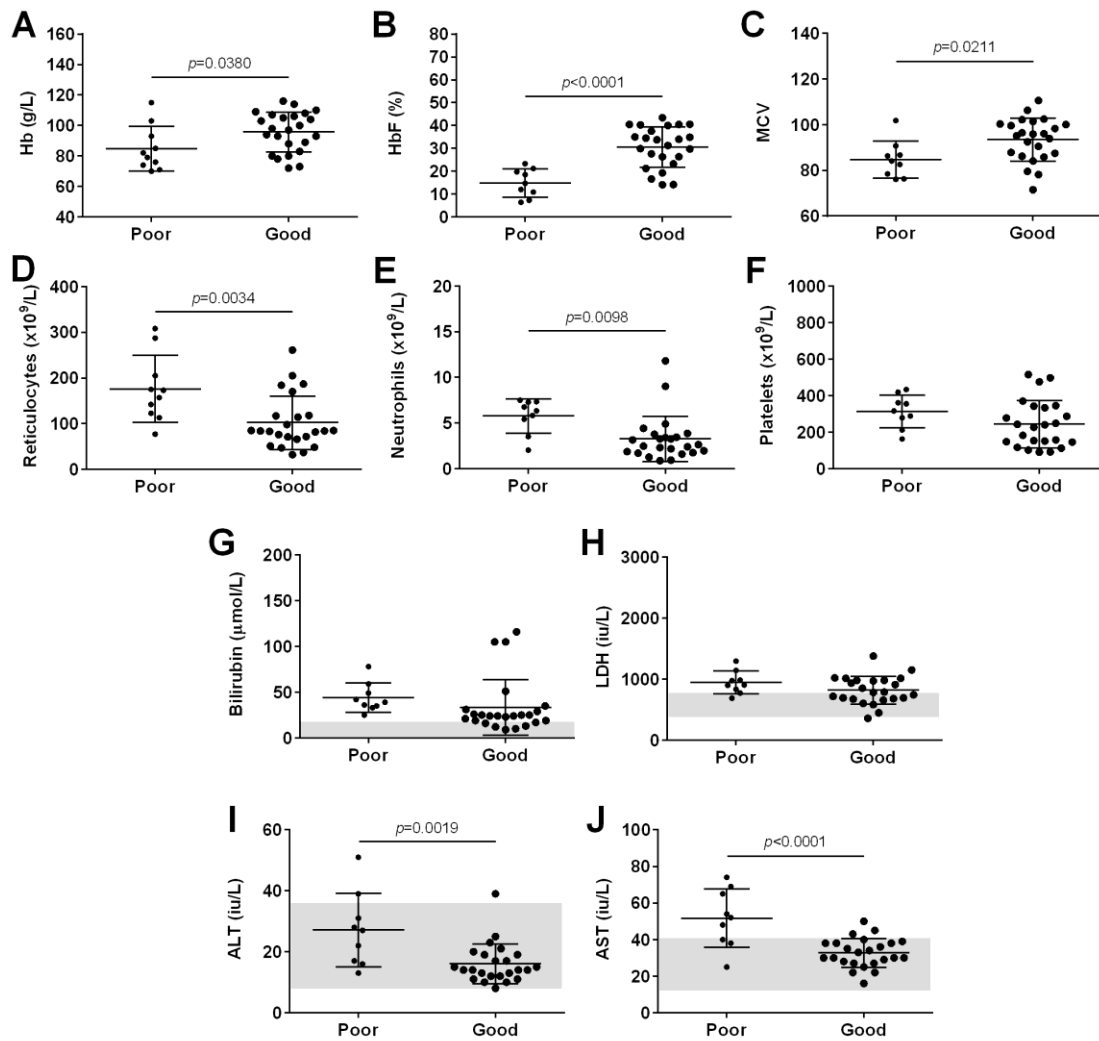
262

263 **Figure 1 Hydroxyurea (HU) improves haematological parameters.** Increased Hb (A), increased HbF (B), increased MCV (C) and decreased reticulocytes (D) were observed post-treatment  
 264 irrespective of dose achieved, although treating with  $\geq 26$ mg/kg further improved HbF, MCV and reticulocyte count. Treatment with  $\geq 26$ mg/kg also resulted in decreased neutrophils (E) and  
 265 platelets (F). Lines indicate mean  $\pm$  SD, statistics by paired t-test for pre/post treatment comparison and by t-test for dose comparison. Dose achieved did not correlate with patient age (G),  
 266 dotted line indicates 26mg/kg threshold used for dose comparison.



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268 **Figure 2 Hydroxyurea (HU) improves control of haemolysis.** Bilirubin (A) and lactate dehydrogenase (LDH; B) were reduced in children receiving  $\geq 26\text{mg/kg}$  hydroxyurea. Alanine amino  
 269 transferase (ALT) remained stable irrespective of dose (C) and aspartate amino transferase (AST) was reduced in children receiving  $\geq 26\text{mg/kg}$  (D). Lines indicate mean  $\pm$  SD, statistics by  
 270 paired *t*-test for pre/post treatment comparison and by *t*-test for dose comparison. Grey shading indicates normal reference range for each parameter measured

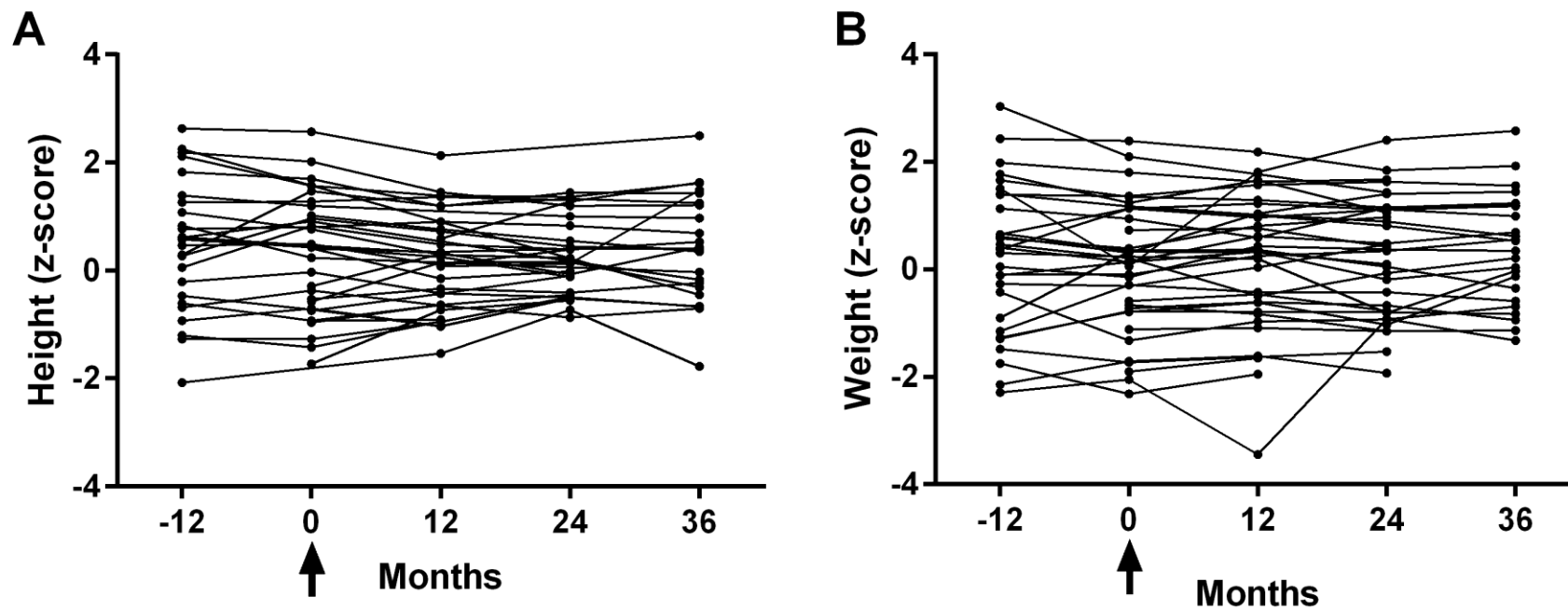


271

272 **Figure 3 Effects of good adherence to therapy.** Good adherence to therapy had a significant effect on Hb (A), HbF (B), MCV  
 273 (C), reticulocyte count (D) and neutrophil count (E) but not on platelet count (F). No significant effects were recorded in  
 274 bilirubin (G) or LDH (H) but ALT (I) and AST (J) were significantly reduced by good adherence to therapy. Lines indicate  
 275 mean  $\pm$  SD, statistics by t-test, grey shading represents normal reference range for each parameter (G-J).

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278 *Figure 4 Growth rates in children following hydroxyurea therapy. Height (A) and weight (B) were maintained in children treated with hydroxyurea. Arrow indicates commencement of*  
 279 *hydroxyurea therapy. Data shown is height for age (A) and weight for age (B) z-score calculated using CDC growth chart[19]*

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