# Fetal haemoglobin and early manifestations of homozygous sickle cell disease

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**Abstract**

**The relevance of fetal haemoglobin (HbF) concentration to the development of early clinical manifestations of homozygous sickle (SS) disease has been investigated by examining the time to first occurrence and the proportional hazard of these complications in three groups of the HbF distribution at age 5 years. HbF was significantly related to dactylitis, painful crises, acute chest syndrome, and acute splenic sequestration. The relationship suggested that a critically low HbF concentration increased the risk, little difference in risk occurring between the medium and high HbF groups. The abdominal painful crisis and hypersplenism were not related to HbF concentration suggesting that the degree of sickling may not be important in their genesis. Parental education on acute splenic sequestration should be focused on children with HbF concentrations in the lowest part of the HbF distribution for age.**

One factor contributing to the highly variable natural history of homozygous sickle cell (SS) disease appears to be the concentration of fetal haemoglobin (HbF). High concentrations are generally associated with mild clinical courses in patients of African origin[1] and also characterise the relatively benign disease observed in the eastern province of Saudi Arabia[2] and central India.[3] However, some patients with high HbF values have severe manifestations and others with low values may be mildly affected, casting doubt on the clinical relevance of HbF concentrations.[4] A Jamaican study of SS disease in the first two years of a neonatally defined cohort has reported that low concentrations of HbF were significantly related to the early appearance of splenomegaly, and a greater incidence of dactylitis and acute splenic sequestration.[5] Observations in this study have now been extended to a median age of 12 years and to other clinical complications.

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## Patients and methods

Patients participated in a cohort study of sickle cell disease based on a cord blood screening programme at the main government maternity hospital (Victoria Jubilee Hospital) in Kingston, Jamaica. Between mid-1973 and late 1981, a total of 100 000 infants were screened with the detection of 314 cases of SS disease. Of these, 308 were located and recruited to cohort clinics operated by the staff of Medical Research Council Laboratories at the University Hospital of the West Indies. Routine appointments were given at intervals of one month up to six months, two months to one year, and three months thereafter and children were encouraged to attend at any time if sick. The study group was confined to 223 subjects (119 male, 104 female) who had survived beyond 4·5 years and in whom adequate HbF estimations and clinical data were available. Of the 85 subjects excluded, 41 had died or emigrated before 4·5 years, and 44 had insufficient HbF values or clinical data. The latter group included patients with more than one year of default in whom it was assumed that recall would be inaccurate. Patients were aged 4·7–17·4 years at the time of the study and median follow up was 12 years.

The diagnosis of SS disease was based on criteria previously described.[6] Blood samples were taken by venepuncture at yearly intervals on the child's birthdate or when clinically indicated. Standard haematological methods were used. HbF was determined by an alkali denaturation method after making standard haemolysates.[7]

The relationship between HbF concentration and clinical events was investigated by dividing patients into three groups on the basis of HbF value at age 5 years. HbF values were available within six months of this target age in 223 subjects and the distribution was derived separately for the two sexes and arbitrarily divided into three groups of approximately equal size representing the low, medium, and high portions of this distribution at age 5 years (table 1). To ascertain that the HbF group at age 5 years was characteristic of an individual subject, the assignment to HbF group was compared in a subset of patients with HbF concentrations at both 5 and 10 years, and showed that the same HbF group assignments occurred in 65/83 (78%) males and in 61/83 (73%) females. All other subjects except one

*Table 1 Concentrations of HbF (%) defining HbF groups in both sexes at 5 and 10 years of age*

| *HbF group* | *Males* | | *Females* | |
|---|---|---|---|---|
| | *No of patients* | *Defining concentration* | *No of patients* | *Defining concentration* |
| Age 5 years: | | | | |
| Low | 40 | ≤4·8 | 36 | ≤6·1 |
| Medium | 40 | 4·9–9·7 | 34 | 6·2–10·3 |
| High | 39 | ≥9·8 | 34 | ≥10·4 |
| Total | 119 | | 104 | |
| Age 10 years: | | | | |
| Low | 26 | ≤2·4 | 30 | ≤3·8 |
| Medium | 28 | 2·5–6·3 | 27 | 3·9–7·4 |
| High | 29 | ≥6·3 | 26 | ≥7·5 |
| Total | 83 | | 83 | |

deviated only to an adjacent HbF group. A similar analysis has also confirmed a close relationship between HbF group assignments at six months and two years.[5]

Clinical events were defined as follows. Dactylitis referred to an acute, painful, non-pitting swelling of the hands, feet, fingers, or toes with no evidence of infection. The painful crisis was defined as an episode of bone pain at two or more sites of sufficient severity to interfere with function, two sites being specified to avoid confusion with localised avascular necrosis of bone. The abdominal painful crisis referred to localised or generalised abdominal pain and tenderness, but without the characteristics of other medical or surgical conditions. Acute chest syndrome was associated with pleuritic pain, fever, cough, respiratory distress, and clinical or radiological signs of pulmonary infiltration. Acute splenic sequestration was defined according to the expanded definition of Topley *et al* involving acute splenic enlargement,[8] a fall in haemoglobin concentration of at least 20 g/l, and evidence of increased bone marrow activity. Hypersplenism applied to sustained splenic enlargement of 4 cm or more associated with haemoglobin concentrations below 65 g/l, and platelet counts below $260\times 10^9$/l on at least two occasions six months or more apart.

The incidence of these clinical events in the two sexes in the different HbF groups was initially examined by the survival curve method, which allows for changes in the population at risk from death, default, or emigration, and the relative risks compared by the log rank test. Significance of the association between clinical events and HbF group was assessed by the $\chi^2$ test for trend. All data were used in the survival analysis, although the figures presented were truncated to 10 years, to avoid the misleading effect on the survival curves of few remaining older patients.

As certain sex differences in the relationship between HbF and clinical features emerged from survival curve analysis and as the HbF distribution differed in the two sexes, these relationships were further investigated by proportional hazards regression using generalised linear interactive modelling (GLIM) and the method described by Aitkin *et al*.[9] This method has the advantage over conventional survival analysis of simultaneous adjustment for a number of potential confounding factors. For this analysis the entire study population was divided into three equal sized groups on the basis of HbF concentration at age 5 years: group I, HbF <5·4%; group II, HbF 5·4–9·7%; group III, HbF ≥9·8%. The factors entered into the model were HbF, sex, and the HbF-sex interaction.

## Results

The relationships between clinical events and HbF group as assessed by survival curve analysis are shown graphically in figs 1–4 and their significance summarised in table 2.

Acute chest syndrome had occurred in 186 patients. Age at first occurrence was significantly related to HbF in both sexes and did not differ between the sexes. The survival curve data (fig 1) suggested that the group with low HbF was particularly prone with less difference between the medium and high HbF groups.

Dactylitis occurred in 113 patients. The trend with HbF was highly significant in males (fig 2A) but although the females in the low HbF

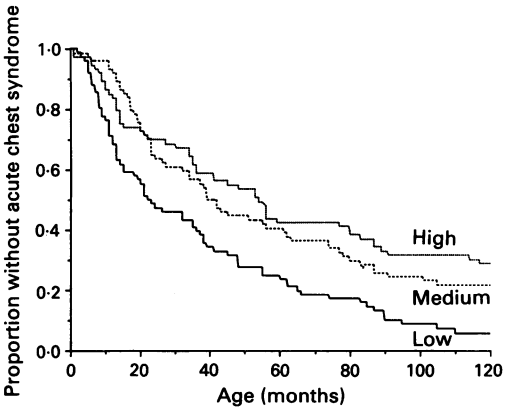


*Figure 1 Survival curve analysis of the first occurrence of the acute chest syndrome in subjects of both sexes according to HbF groups.*

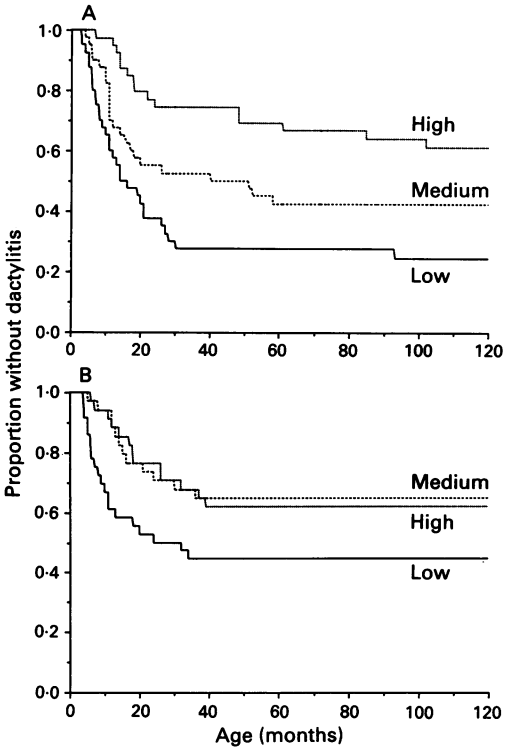


*Figure 2 Survival curve analysis of the first occurrence of dactylitis according to HbF group in (A) males and (B) females.*

*Table 2 Relationship of clinical events with HbF group assessed by $\chi^2$ test for trend on survival curve analysis*

| Event | Males | | Females | |
|---|---|---|---|---|
| | $\chi^2$ | p Value | $\chi^2$ | p Value |
| Acute chest syndrome | 8·07 | 0·005 | 6·30 | 0·012 |
| Dactylitis | 14·54 | <0·001 | 3·45 | 0·063 |
| Painful crisis | 5·69 | 0·017 | 0·58 | 0·445 |
| Acute splenic sequestration | 14·13 | <0·001 | 2·78 | 0·095 |
| Hypersplenism | 0·31 | 0·580 | 0·03 | 0·869 |
| Abdominal painful crisis | 0·83 | 0·363 | 1·41 | 0·236 |

group were affected earlier and more frequently (fig 2B), the trend was not significant in females.

Painful crises occurred in 157 patients. The relationship with HbF group was significant in males (fig 3A) but not in females (fig 3B).

Acute splenic sequestration occurred in 88 patients. The relationship with HbF concentration was significant in males (fig 4A) but not in females (fig 4B). As expected, most episodes occurred before 3 years of age and of those episodes occurring after 3 years, all but one were in the high HbF group.

Hypersplenism occurred in 32 patients, and there was no relationship with HbF concentration in either sex. Abdominal painful crisis occurred in 123 patients, and was not influenced by HbF group in either sex.

The apparent sex difference in the significance of HbF with some complications could have resulted from the greater proportion of males with low HbF values if a critically low concentration of HbF were an important determinant. The results of proportional hazard analysis support this as expressing the risk of complications in HbF groups II and III in relation to group I (table 3) show significantly lower risk in the higher HbF groups for dactylitis, painful crisis, acute chest syndrome, and acute splenic sequestration. Furthermore, adding sex to the model did not improve the fit indicating that these relationships were true independent of sex. Proportional hazard analysis also confirmed the lack of any relationship between HbF and abdominal painful crisis or hypersplenism.

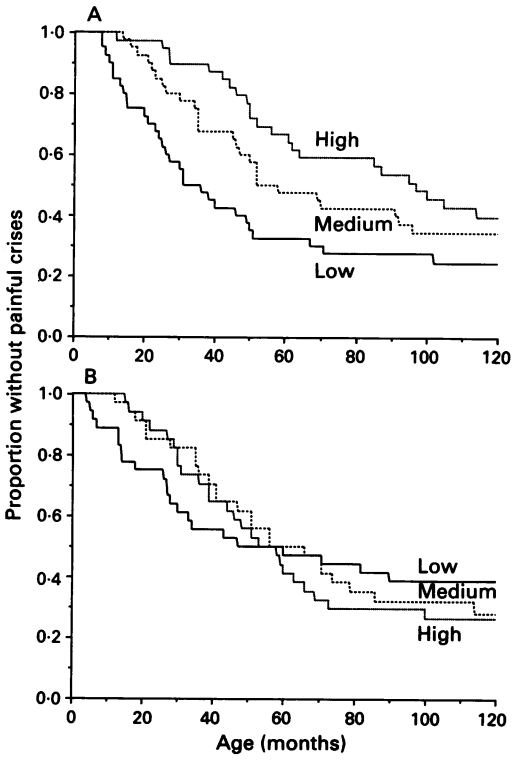


*Figure 3 Survival curve analysis of the first occurrence of painful crisis according to HbF group in (A) males and (B) females.*

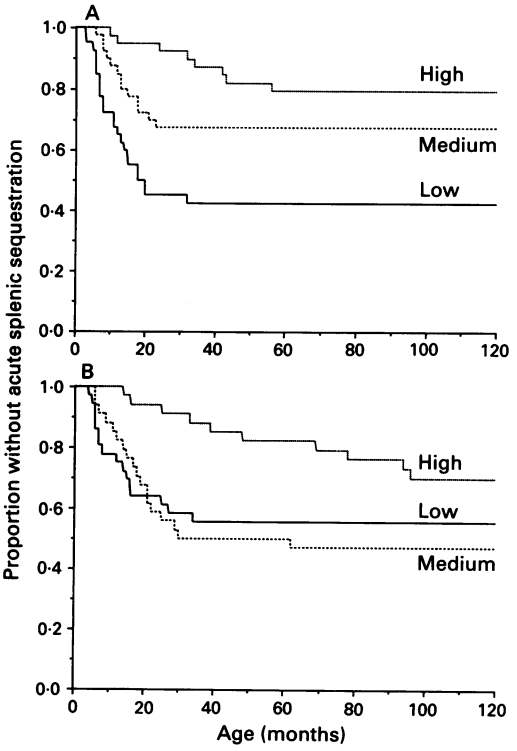


*Figure 4 Survival curve analysis of the first occurrence of acute splenic sequestration according to HbF group in (A) males and (B) females.*

*Table 3 Hazard rates for clinical events according to HbF grouping expressed relative to group I (95% confidence intervals)*

| *Event* | *Group II (HbF 5·4–9·7%)* | *Group III (HbF ≥9·8%)* |
|---|---|---|
| Acute chest syndrome | 0·55 (0·39 to 0·78) | 0·48 (0·34 to 0·69) |
| Dactylitis | 0·39 (0·25 to 0·61) | 0·32 (0·20 to 0·52) |
| Painful crisis | 0·59 (0·40 to 0·88) | 0·69 (0·47 to 1·01) |
| Acute splenic sequestration | 0·63 (0·39 to 1·02) | 0·31 (0·18 to 0·55) |
| Hypersplenism | 0·62 (0·25 to 1·53) | 0·83 (0·36 to 1·88) |
| Abdominal painful crisis | 0·96 (0·62 to 1·49) | 0·96 (0·62 to 1·50) |

## Discussion

Previous observations in the cohort study showed that patients with low concentrations of HbF were more prone to develop dactylitis and acute splenic sequestration.[5] The present study has shown that low HbF is also a risk factor for painful crisis and acute chest syndrome. The relevance of HbF to clinical features appears to conflict with observations by Powars *et al* in Los Angeles who initially failed to find a relationship between HbF concentration and any clinical event except stroke,[4] although they later postulated that a critical concentration of HbF may protect against certain complications of the disease.[10]

Several differences in the Jamaican and American studies may have contributed to the apparently different conclusions. The present study was confined to survival curve analysis, which assesses only the timing of the first event and not the frequency of subsequent events within individuals. The Jamaican study has used clearly defined end points, which have been consistently applied from the onset of the cohort study. Furthermore the Jamaican study has used only prospectively recorded data in contrast to the American study that used inevitably less accurate retrospective data.

Finally the Jamaican population was a representative sample in contrast to the American group who were predominantly symptomatically acquired. For these reasons it seems justified to conclude that the Jamaican observations are less prone to bias.

Children in the lowest third of the HbF distribution at age 5 years were more prone to the acute chest syndrome, dactylitis, painful crises, and acute splenic sequestration. As there was a close correlation between HbF groupings at six months and two years[5] and between age 5 and 10 years, it seems reasonable to assume that the position within an HbF distribution is characteristic for an individual child. Placement within the HbF distribution may therefore be of prognostic importance for these complications and prophylactic or education programmes may be devised accordingly. For example education on parental detection of acute splenic sequestration[11] may be focused in high risk groups.

The apparent conflict between data from the survival curve analysis that showed some sex differences and that from proportional hazard analysis that did not, could be explained if a HbF value below a critical concentration was necessary as a risk factor for these complications and that this was achieved in males because of their generally lower HbF distribution.[12] This concept was supported by proportional hazard analysis that indicated that for dactylitis, painful crises, and acute chest syndrome, there did appear to be a critical concentration of HbF below which the hazard was increased.

The significant relationship between painful crisis and HbF value may appear at variance with an earlier study that failed to show a relationship between HbF and painful crisis frequency in either sex.[13] However, the end points of the two studies were different, the present study examining the age of onset of painful crises and the earlier study addressing painful crisis frequency. Both dactylitis and the painful crisis result from avascular necrosis of bone marrow and a similar behaviour to risk factors might have been anticipated. Avascular necrosis of bone marrow is likely to be influenced by both oxygen supply and demand of the expanded bone marrow. HbF concentration could influence supply by its effect on sickling and vaso-occlusion but could also influence demand if subjects with lower HbF concentrations had more rapid haemolysis and greater erythropoietic expansion.

Abdominal painful crisis and, to a lesser extent, hypersplenism appeared unrelated to HbF group. The pathology of the abdominal painful crisis remains unknown but the lack of a relationship with HbF suggests that this complication is not closely related to vaso-occlusion. Low concentrations of HbF have been previously associated with the early appearance of splenomegaly[5] and high concentrations of HbF influence the persistence of palpable splenomegaly.[14] There was the suggestion of a trend between hypersplenism and HbF group in the present study but this failed to reach significance possibly because of the relatively small numbers of subjects with this complication.

The protective effect of HbF is believed to be twofold. High concentrations of HbF infer lower concentrations of sickle cell haemoglobin (HbS) within the cell but HbF molecules also participate less readily in HbS polymer formation. As a result there is an inverse relationship between HbF value and counts of irreversibly sickled cells.[14 15] The present study confirms that high and moderate concentrations of HbF determine a lower frequency and a later onset of the acute chest syndrome, dactylitis, painful crises, and acute splenic sequestration.


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