

Normal haemoglobin electrophoretic pattern in a patient with sickle cell disease and end stage renal failure

Chronic renal failure is an important cause of morbidity and mortality in older patients with sickle cell disease,¹ and its onset is often heralded by a progressive fall in steady state haemoglobin concentration.² The renal failure is probably multifactorial, and recent work has suggested that an associated reduction in erythropoietin activity may play a particularly important part in the development of anaemia.^{3,4} It is also well recognised that regular blood transfusion in sickle cell disease in the absence of renal impairment suppresses endogenous erythropoiesis.

We report a patient with sickle cell anaemia who developed chronic renal failure and a regular requirement for blood transfusions. This combination of factors produced such a profound degree of bone marrow erythropoietic suppression that the presence of sickle haemoglobin could no longer be detected by routine electrophoretic procedures.

In 1975 a 27 year old woman from Montserrat travelled by air to London and shortly after arrival presented with limb and back pains. Examination showed that she had anaemia, jaundice, heart failure with a blood pressure of 170/100 mm Hg and bilateral chronic leg ulceration. Haematological investigation showed haemoglobin to be 6.0 g/dl, white cells $11.6 \times 10^9/l$ (differential count normal), platelet count $375 \times 10^9/l$, and a reticulocytosis of 34%. Hb SS disease was diagnosed by a positive sickle solubility test and the presence of a major band in the position of Hb S with a faint band in the position of Hb F on haemoglobin electrophoresis (cellulose acetate, pH 8.6, and agar gel, pH 6.8). Hb F value was 5.5% of the total haemoglobin. Serum urea and creatinine concentrations were normal.

Eight years later mild renal impairment had become apparent which over the next two years progressed to chronic renal failure associated with anaemia requiring regular blood transfusion. Investigation now showed a serum urea concentration of 65.4 mmol/l, creatinine concentration of 1003 $\mu\text{mol/l}$, and creatinine clearance 2 ml/minute. Urinary protein output was 1.8 g/24 hour; she was abacteruric and a renal ultrasound scan was normal. A renal biopsy specimen showed glomerulosclerosis, severe tubular atrophy, diffuse interstitial fibrosis, and increased amounts of haemosiderin in tubular epithelial cells. The biopsy specimen showed no evidence of amyloid or hypertensive change. There was no evidence of

immunologically mediated disease on electron microscopical examination or immunofluorescence. Her renal failure was successfully managed by continuous ambulatory peritoneal dialysis (CAPD).

Haematological investigation seven weeks after blood transfusion now showed a haemoglobin concentration of 5.7 g/dl, a reticulocyte count of <2%, with normal white cell and platelet counts. A sickle solubility test was negative and haemoglobin electrophoresis on cellulose acetate and agar gel showed only Hb A. Analysis of the absorbance of haemoglobin eluted from CM cellulose chromatography showed α and β^A peaks but no β^S peak (figure). Examination of the rate of incorporation of ³H-leucine into the globin of reticulocytes from the patient with fractionation of the globin chains by CM cellulose chromatography, however, showed incorporation into only α and β^S fractions, consistent with synthesis of only Hb S (figure). The α/β globin biosynthesis ratio was normal at 0.997. Analysis of peripheral blood DNA using the restriction enzyme MST II showed only the 1.3 Kb gene fragment, thus confirming the presence of homozygous sickle cell disease (Hb SS). Ferrokinetic studies showed a normal ⁵⁹Fe plasma clearance T_{50} of 94 minutes (normal range 60–140) with a plasma iron concentration of 32 $\mu\text{mol/l}$ (normal range 14–29). Red cell utilisation was greatly reduced at 2% on day 6 (normal maximum utilisation is 70–80% by day 10–14). A bone marrow aspirate showed severely reduced erythropoietic activity with greatly increased reticulo-endothelial iron but absent erythron iron. Serum assay for human parvovirus Ig M

antibody showed no rise, thus making recent infection unlikely to be the cause for the aplasia.

In view of our findings a negative sickle solubility test with a normal haemoglobin electrophoretic pattern may need to be anticipated in patients with homozygous sickle cell disease, end stage renal failure, and a transfusion requirement.

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References

- 1 Thomas AN, Pattison C, Sergeant GR. Causes of death in sickle cell disease in Jamaica. *Br Med J* 1982;285:633–5.
- 2 Morgan AG, Gruber CA, Sergeant GR. Erythropoietin and renal function in sickle cell disease. *Br Med J* 1982;285:1686–8.
- 3 Sergeant GR. *Sickle cell disease*. Oxford University Press, 1985.
- 4 Eschbach JW, Adamson JM. Anaemia of end stage renal disease (ESRD). *Kidney Int* 1985;28:1–5.

Four hour rapid urease test (RUT) for detecting *Campylobacter pylori*: is it reliable enough to start treatment?

Detection of *Campylobacter pylori* in antral or duodenal biopsy specimens usually entails histological or microbiological methods that may not produce a result for seven days. Langenberg *et al* described the unusual characteristic of rapid urea hydrolysis by *Campylobacter pylori* that indicated the presence of pre-formed urease.¹ McNulty and Wise reported a rapid urease test (RUT) capable of detecting the presence of *Campylobacter pylori* on the same day as endoscopy.² An evaluation of the commercially available CLO-Test has also recently been reported.³ More recently, however, Das *et al* cast doubt on the reliability of this test by reporting specificity at 86% and sensitivity at only 59%.⁴ In a small series we previously described that the specificity and sensitivity of RUT was 100% and 81%, respectively.⁵

We now report our results on a modifica-

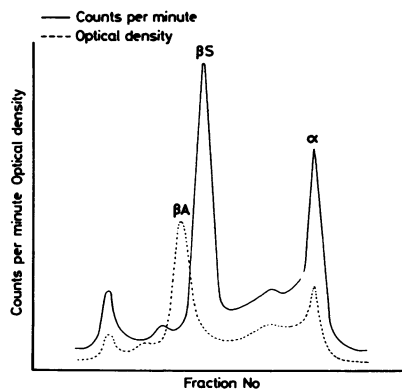


Figure CM cellulose chromatographic fractionation of globin: — — — — —: absorbance at 280 nm; — — — — —: incorporated ³H-leucine.

tion of the four hour RUT for the detection of *Campylobacter pylori*. Two hundred and fifty six mucosal biopsy specimens were taken from the gastric antrum and duodenum for a four hour RUT, histological examination, and culture. Histology specimens were stained with Giemsa stain and examined for the presence of *Campylobacter pylori*. Specimens for culture were placed in 0.5 ml of 20% glucose, homogenised, and a drop taken for immediate Gram staining and the remainder placed on blood agar and campylobacter medium with Skirrow supplement. Plates were incubated in a microaerophilic environment at 37°C for six days. *Campylobacter pylori* colonies were examined for the presence of oxidase and urease and confirmation was obtained by Gram stain of the culture. One biopsy specimen (about 0.02 mg) was homogenised in 0.9% saline 0.5 ml, inoculated into 2% urea broth, incubated at 37°C, and examined after four hours. There was an excellent correlation between this modified four hour RUT, histological results, and culture. The table gives results of all biopsy specimens showing four hour RUT specificity at 100% and sensitivity at 89%. Specificity and sensitivity for antral biopsy specimens was 100% and 97% respectively.

False negative results for four hour RUT may be due to low numbers of bacteria within the specimen. We serially diluted *Campylobacter pylori* and inoculated dilutions into RUT broth which was incubated at 37°C and 50°C. We observed that the RUT test became positive within one hour at bacterial concentrations of $> 10^7$ colony forming units/ml (cfu/ml) when incubated at 37°C and of $> 10^6$ cfu/ml at 50°C. All biopsy specimens in which *Campylobacter pylori* could be detected showed evidence of acute gastritis or duodenitis.

These results indicated that the specificity of our test was higher than the CLO-Test (100% v 97%) and that a positive four hour RUT is sufficient evidence to start treatment if appropriate. Preliminary results suggest that incubation at a higher temperature (50°C) may further enhance the sensitivity of the four hour RUT.

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Results of antral and duodenal biopsy specimens

	Histology	Culture	Four hour RUT
Positive	91	63	81
Negative	165	191	175
Total	256	254	256

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References

- Langenberg ML, Tytgat GNJ, Schipper MEI, Rietra PJGM, Zanen HC. Campylobacter-like organism in the stomach of patients and healthy individuals. *Lancet* 1984;i:1348.
- McNulty CAM, Wise R. Rapid diagnosis of Campylobacter-associated gastritis. *Lancet* 1985;i:1443-4.
- Borromeo M, Lambert JR, Pinkard KJ. Evaluation of "CLO-Test" to detect Campylobacter pyloridis in gastric mucosa. *J Clin Pathol* 1987;40:462-3.
- Das SS, Bain LA, Karim QN, Coelho LG, Baron JH. Rapid diagnosis of Campylobacter pyloridis infection. *J Clin Pathol* 1987;40:701-2.
- Vaira D, Holton J, Falzon M, Ahmed A, Cairns SR, Salmon PR. Comparative study of 4-hour rapid urease test (RUT) to culture and histology for the detection of Campylobacter pyloridis (CP) in gastric and duodenal biopsies. *Gut* 1987;28:A1406.

Des-gamma-carboxyprothrombin and hepatoblastoma

Increased serum α fetoprotein is used as a diagnostic marker of hepatoblastoma in children, but in some cases, the serum α fetoprotein is normal.¹ We wanted to know if the des-gamma-carboxyprothrombin concentration (DCP), a newly described marker of hepatocellular carcinoma in adults,² could be used in the diagnosis of hepatoblastoma.

Raised plasma concentrations of DCP, the des-gamma-carboxylated form of prothrombin (factor II), are seen in vitamin K deficiency, in patients taking oral anticoagulants, and in most patients with hepatocellular carcinomas.^{2,3} We assayed DCP value by a previously described method³ using staphylocoagulase (upper limit of normal, 15 mU/ml) in three children with histologically confirmed hepatoblastoma. None had received treatment. DCP, α fetoprotein, and vitamin K concentrations

are shown in the table. The prothrombin time and serum vitamin K concentrations (normal range 0.2-0.8 ug/l) were normal in all three children.

The increased plasma DCP concentration in these three patients was not due to vitamin K deficiency, and presumably results from the same (unknown) mechanism, observed in adult hepatocellular carcinoma. We found normal DCP values in other liver diseases of children usually associated with an increased α fetoprotein concentration: hereditary tyrosinemia (n = 2), giant cell hepatitis (n = 4), fulminant hepatitis (n = 1), and neonatal hepatic necrosis with hepatic regeneration (n = 2).

Other studies are required to determine the respective value of DCP and α fetoprotein assays as markers of hepatoblastoma in diagnosis and treatment, but we suggest that this marker may be useful in rare cases of hepatoblastomas with normal serum α fetoprotein. It should be noted that in adults with hepatic carcinoma, there is a weak correlation between increased DCP and increased α fetoprotein, the two markers thus being to some extent complimentary.

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References

- Bergsmand CG. Alpha-fetoprotein in pediatrics. *Acta Paediatr Scand* 1986;75:1-9.
- Liebman HA, Furie BC, Tong MJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. *N Engl J Med* 1984;310:1427-31.
- Soulier JP, Gozin D, Lefrère JJ. A new method to assay des-gamma-carboxyprothrombin. Results obtained in 75 cases of hepatocellular carcinoma. *Gastroenterology* 1986;91:1258-62.

Table α fetoprotein, DCP, and vitamin K concentrations in hepatoblastoma

	α -fetoprotein (U/ml)	DCP (mU/ml)	Vitamin K (μ g/l)
6 month old male infant	335.000	400	0.21
13 year old girl	600.000	70	0.30
6 year old girl	450.000	110	0.40