

Rapid interphase FISH diagnosis of trisomy 18 on blood smears

SIR,—Dr Bos, Dr Wolstenholme, and their colleagues (April 11, p 913; June 6, p 1416) comment on the importance of obtaining rapid chromosome diagnosis in babies with life-threatening malformations that are compatible with trisomy 18, since this is an essential part of the clinical evaluation with respect to urgent surgical treatment. We agree with this notion and have developed an alternative strategy, using a new fluorescence in-situ hybridisation (FISH) technique for diagnosis of trisomy 18 in non-dividing (interphase) cells from direct blood smears without intervening cell culture.

This situation is exemplified by the case of dizygous twins born prematurely at 32 weeks' gestation to a 33-year-old primigravida. Twin 1 was small for dates (birthweight 1.2 kg) and needed ventilation for recurrent apnoea. He also proved to have a tracheo-oesophageal fistula. On day 2 he developed acute abdominal distension with fluid on radiography. At this stage, a clinical diagnosis of trisomy 18 was made on the basis of his other features. Urgent decisions about transferring the baby to a regional surgical unit were necessary. Blood was taken for chromosome analysis.

1–2 drops of blood were smeared on to slides. The slides were dried, fixed for 30 min in 3/1 methanol/acetic acid, and dehydrated through an ethanol series. A directly fluoresceinated chromoprobe specific for the 18 centromere was used. Hybridisation was done overnight at 37°C. Slides were processed by in-situ hybridisation techniques without the need for signal amplification¹ and examined by fluorescence microscopy. 59 of 163 interphase cells examined showed three spots compared with 2 of 104 cells with three spots in a control. The diagnosis of trisomy 18 was confirmed by conventional 48 h lymphocyte culture. After confirmation of the diagnosis active treatment was withdrawn. Twin 2 is normal and healthy.

We have since modified the FISH technology, applying a 30 min fixation of the blood smear, followed by 6 min dehydration and hybridisation for 1 h, plus post-hybridisation washes of 30 min. Thus slides are ready for microscopy after about 2 h and diagnosis is available within 3 h of taking the blood smear. The efficacy of this approach is high (to be reported elsewhere). Additionally, the same approach is applicable to other trisomies such as trisomy 21 Down's syndrome.

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1. Collins C, Kuo WL, Segraves R, Fuscoe J, Pinkel D, Gray JW. Construction and characterization of plasmid libraries enriched in sequences from single human chromosomes. *Genomics* 1991; 11: 997–1006.

Helicobacter pylori infection in early infancy

SIR,—In western countries, the frequency of *Helicobacter pylori* infection is low in children and increases with age.¹ In France, less than 1% of children were serologically positive before age 6.² Most of the epidemiological reports in young infants are based on serological studies, suggesting the possibility of *H pylori* infection in infancy, especially in developing countries.³ However, there are a few reports of *H pylori* infection confirmed by presence of organism in gastric mucosa in young children.⁴ The youngest child reported was 2 years old.⁵ We report the first cases of *H pylori* infection in earlier infancy.

Over 1 year, *H pylori* infection was investigated in 67 children among 320 undergoing gastrointestinal endoscopy in our institution. 22 (33%, mean age 11.2 years) were infected, defined by presence of *H pylori* in antral mucosa by Giemsa staining and/or bacteriology. 3 (14%) were less than 6 months old (table). All the infants were white and 2 lived in poor socioeconomic conditions. Serological testing was only done for patient 2 and his mother, and

CHARACTERISTICS OF 3 INFANTS WITH *H PYLORI* INFECTION

	1*	2	3
Age (mo)	2.5	2.5	5.5
Signs	Crying, vomiting	Haematemesis, vomiting	Failure to thrive
Endoscopy	Gastric ulcer	Oesophagitis	Normal
Histology	Gastritis	Normal	Gastritis
Giemsa	+	—	+
Culture	—	$3 \times 10^3/g$	$1.5 \times 10^7/g$

*Patient 1 had had gastro-oesophageal reflux.

was negative. Patient 1 received amoxicillin and antacid for 4 weeks; 1 month later, gastric mucosa was normal and *H pylori* was not found.

Thus *H pylori* infection can occur in infants under 6 months, even in developed countries and particularly in children living in poor conditions. Nodular gastritis is common in *H pylori* infected children⁶ and occurred in 40% of our 67 patients but was absent in the 3 infants. This finding suggests that nodules are not associated with very early infection but with more "chronic" infection in children. The reason why *H pylori* infection in infancy can be associated either with acute gastric lesions or with normal gastric mucosa, the mode of transmission, and the course of *H pylori* infection in infancy remain unknown. Systematic investigation for this organism in infants undergoing upper gastrointestinal endoscopy and follow-up should answer these questions.

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- Taylor DN, Blaser MJ. The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev* 1991; 13: 42–59.
- Megraud F, Brassens-Rabbe MP, Denis F, et al. Seroepidemiology of *Campylobacter pylori* infection in various population. *J Clin Microbiol* 1989; 25: 1870–73.
- Perez-Perez GI, Taylor DN, Bodhidatta L, et al. Seroprevalence of *Helicobacter pylori* infection in Thailand. *J Infect Dis* 1990; 161: 1237–41.
- Prieto G, Polanco I, Larrauri J, Rota L, Lama R, Carresco S. *Helicobacter pylori* infection in children: clinical endoscopic, and histologic correlations. *J Pediatr Gastroenterol Nutr* 1992; 14: 420–25.
- Czinn SI, Carr H. Rapid diagnosis of *Campylobacter pyloridis*-associated gastritis. *J Pediatr* 1987; 110: 569–70.
- Bujanover Y, Konikoff F, Baratz M. Nodular gastritis and *Helicobacter pylori*. *J Pediatr Gastroenterol Nutr* 1990; 11: 41–44.

Isotretinoin and the axial skeleton

SIR,—Synthetic retinoids, including isotretinoin, have been implicated in the induction of hyperostotic changes in the axial and appendicular skeleton.^{1–3} We have prospectively reviewed⁴ radiographs from a random sample of 269 patients in a controlled trial to evaluate the chemopreventive effectiveness of chronic, very low dose isotretinoin (about 0.14 mg/kg per day for 3 years) in basal cell carcinoma. This regimen was ineffective in preventing the occurrence of new basal cell carcinomas.⁵ However, our review showed that, compared with the placebo group, significantly more patients in the isotretinoin group showed progression of existing vertebral hyperostoses (40.3% vs 18.5%, $p < 0.001$) and new hyperostotic vertebral involvement (8.6% vs 1.5%, $p = 0.015$) of the cervical and thoracic spine.⁴ We have now done a post-treatment radiographic review to determine whether the calcifications and hyperostoses induced by isotretinoin regressed, and whether the overall effect of isotretinoin on the axial skeleton continued to be manifest off-treatment.

109 patients were selected from the original 269 on the basis of having lateral cervical and thoracic radiographs taken 10–24 months after the 36 month treatment ended. We followed our earlier procedures.⁴ 36 month and post-treatment films for each patient were read side-by-side by the radiologist (R. F. K.) who was masked to treatment assignment. He noted the presence and extent of hyperostotic abnormalities at each vertebral level (C1 to T12) at 36 months, post-treatment progression or regression of existing abnormalities, and the occurrence of new abnormalities at previously unaffected vertebral levels.

The 109 patients were predominantly male (75%) with a median age of 62 years (range 40–75). Baseline characteristics did not differ

NUMBER (%) PATIENTS WITH SPINAL LIGAMENT CALCIFICATION AND/OR VERTEBRAL HYPEROSTOSES DETECTED BY POST-TREATMENT RADIOGRAPHS

Type of change	Isotretinoin (n=52)	Placebo (n=57)
Progression of existing ligament calcification or vertebral hyperostoses	11 (21)	11 (19)*
New ligament calcification or vertebral hyperostoses†	1(2)	3 (5)†

Fisher's exact test: * p = 0.82, † p = 0.62.
†Of previously unaffected vertebral level.

between the treatment and control groups within this subset, or between these patients and the 160 patients who did not have post-treatment radiographs available (data not shown). The mean number of months between 36 month and post-treatment films also did not differ by treatment group (17.5 months, isotretinoin group; 16.6 months, placebo group; p = 0.30).

There was no X-ray evidence that the skeletal changes observed in the 36 month films regressed once treatment ended. Further, there were no significant treatment group differences in the proportions of patients who developed either progression of existing vertebral hyperostoses or new hyperostoses at previously unaffected vertebral levels post-treatment (table). We conclude that, at least in older patients, continuous exposure to isotretinoin may be required for the induction of hyperostotic effects in the axial skeleton and that radiographic monitoring beyond the treatment period may not be necessary.

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- Lawson JP, McGuire J. The spectrum of skeletal changes associated with the long-term administration of 13-cis-retinoic acid. *Skel Radiol* 1987; 16: 91-97.
- Kilcoyne RF. Effects of retinoids in bone. *J Am Acad Dermatol* 1988; 19: 212-16.
- White SI, MacKie RM. Bone changes associated with oral retinoid therapy. *Pharmac Ther* 1989; 40: 137-44.
- Tangrea JA, Kolcoyne RF, Taylor PR, et al. Skeletal hyperostosis in patients receiving long-term, very low-dose isotretinoin. *Arch Dermatol* 1992; 128: 921-25.
- Tangrea JA, Edwards BK, Taylor PR, et al. Long-term therapy with low-dose isotretinoin for prevention of basal cell carcinoma: a multicenter clinical trial. *J Natl Cancer Inst* 1992; 84: 328-32.

Neurological symptoms associated with cyclosporin plus doxorubicin

SIR,—Dr Barbui and colleagues (June 6, p 1421) describe neurological complications of administering doxorubicin to a patient with stage IV Burkitt's lymphoma who had been receiving long-term cyclosporin immunosuppression after heart transplantation.¹ The patient twice went into a coma after administration of doxorubicin, the second episode being fatal. They then tested the hypothesis that doxorubicin and cyclosporin have synergistic deleterious effects on the central nervous system (CNS) by giving rats cyclosporin or vehicle control only for 4 months, after which time the animals were given a single intravenous injection of doxorubicin. Cyclosporin-treated rats had progressive paresis of all four limbs in 17-23 h and died a few hours after the onset of paresis; control rats had no paresis. Doxorubicin concentrations in the brains of the cyclosporin-treated rats were 2-4 times higher than the detection limit of the assay and cerebellar levels were 3-25 times higher. No doxorubicin was detected in the brains of the vehicle-treated animals. While Barbui et al could not explain their findings they urged caution in giving doxorubicin to cancer patients who were on cyclosporin.

Doxorubicin has been associated with acute and even fatal encephalopathy after inadvertent administration intrathecally.^{1,2} This agent is one of many "natural product" anti-cancer drugs that is a substrate for efflux by P-glycoprotein (Pgp).³ Cyclosporin can inhibit Pgp and reverse multidrug resistance (MDR),^{4,5} and this drug at non-immunosuppressive doses is undergoing clinical trials in North America and Europe as a modulator of clinical MDR.⁶ Pgp is expressed on the luminal surfaces of capillary endothelial cells and may have a physiological role as a component of the blood-brain barrier.⁷ Cyclosporin accumulates in the brain, albeit at low levels.⁸ The neurological effects and high brain levels of doxorubicin described by Barbui et al may have been a consequence of cyclosporin inhibiting Pgp in the brain capillary endothelial cells, thereby raising doxorubicin levels in the brain. Neurological problems associated with long-term cyclosporin have been ascribed to hypomagnesaemia due to renal toxicity of the immunosuppressive agent.⁹ However, it is unlikely that the toxicity seen in Barbui's patient or in the rats was due to hypomagnesaemia, because the neurological events were only seen after the administration of doxorubicin. Although clinical trials investigating the modulation of Pgp function in patients' tumour cells use cyclosporin under vastly different conditions from those of immunosuppression, we agree with Barbui et al that investigators should approach the co-administration of doxorubicin and cyclosporin with caution. This concern gains importance in the light of the fact that future clinical trials of MDR modulators will use more potent non-immunosuppressive analogues of cyclosporin, such as SDZ PSC 833.¹⁰

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- Arico M, Nespole L, Porta F, Caselli D, Raiteri E, Burgio GR. Severe acute encephalopathy following inadvertent intrathecal doxorubicin administration. *Med Pediatr Oncol* 1990; 18: 261-63.
- Mortensen ME, Cecalupo AJ, Lo WD, Egorin MJ, Barbui R. Inadvertent injection of daunorubicin with fatal outcome. *Med Pediatr Oncol* 1992; 20: 249-53.
- Busche R, Tummeler B, Riordan JR, Cano-gauci DF. Preparation and utility of a radioiodinated analogue of daunomycin in the study of multidrug resistance. *Mol Pharmacol* 1989; 35: 414-21.
- Slater LM, Sweet P, Stupecky M, Gupta S. Cyclosporin A reverses vincristine and daunorubicin resistance in acute lymphatic leukemia in vitro. *J Clin Invest* 1986; 77: 1405-08.
- Twentyman PR, Wright KA. Chemosensitisation of a drug-sensitive parental cell line by low-dose cyclosporin A. *Cancer Chemother Pharmacol* 1991; 29: 24-28.
- List AF, Spier C, Greer J, et al. Biochemical modulation of anthracycline resistance (MDR) in acute leukemia with cyclosporin-A (CSA). *Proc Am Soc Clin Oncol* 1992; 11: 264.
- Cordon-Cardo C, O'Brien JP, Casals D, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci USA* 1989; 86: 695-98.
- Atkinson K, Boland J, Britton K, Biggs J. Blood and tissue distribution of cyclosporin in humans and mice. *Transplant Proc* 1983; 15: 2430.
- Thompson CB, June CH, Sullivan KM, Thomas ED. Association between cyclosporin neurotoxicity and hypomagnesaemia. *Lancet* 1984; ii: 1116-20.
- Boesch D, Gavériaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loo F. In vivo circumvention of P-glycoprotein mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res* 1991; 51: 4226-33.

Retraction: Effects of interleukin-1 on platelet counts

SIR,—We have become aware that there were some arithmetical errors made in summarising the data for our preliminary report.¹ We believe that the overall conclusions as originally stated are valid. Nonetheless, we would like to retract this paper. We plan to submit the full report of this study to a journal in the future. We very much regret this episode.

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- Towari A, Buhles WC Jr, Starnes HF Jr. Effects of interleukin-1 on platelet counts. *Lancet* 1990; 336: 712-14.