Within 24 h of admission, he worsened with a temperature of 39·4°C and respiratory distress (radiography showed right middle-zone bronchopneumonic infiltrates and mild cardiomegaly). Except for moderate reduction in rigidity, the other features remained unchanged and spasms were frequent. Supplementary oxygen was added but about 12 h later, he had gross haematuria and packed cell volume dropped to 11%; serum urea was elevated at 12·4 mmol/l. Shock rapidly developed (unresponsive to plasma expanders), and the patient lasped into coma and died about 39 h after admission, less than 72 h after the initial symptom. The father had refused blood transfusion and necropsy on religious grounds.

Occasionally no portal of entry can be found and although tetanus has occurred after minor procedures, such as piercing of the ear lobes,[2] it is probably rare after dental procedures. Bleeding into the tooth socket might provide the necessary anaerobic environment. Even though our patient had not received the booster dose at age 5, reasonable amelioration of the severity of tetanus is expected for up to 10 years after the primary course of vaccination. Whereas vaccination and a long incubation period of 2 weeks were favourable factors, this course was unusually severe and rapidly fatal.

SCD and other haemoglobinopathies are not usually associated with an enhanced risk of or a poorer outcome in tetanus. Myoglobinaemia with shock and myoglobinuria, both of which could lead to renal failure, are consequences of severe spasms. Thus intensive care is probably required in the management of tetanus complicating SCD. Unfortunately, such facilities are in short supply in developing countries. Therefore, although the patient we saw is rare, such cases need policy reinforcement or review to ensure that booster doses of tetanus toxoid are received.

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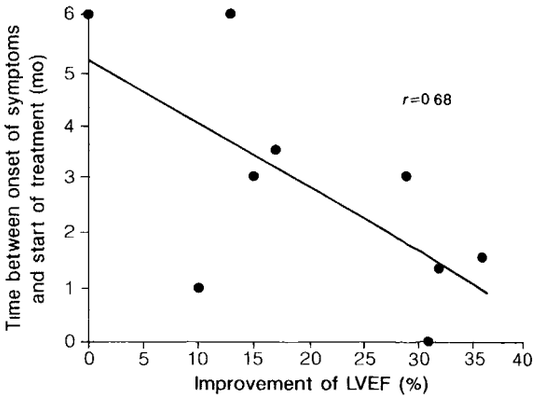
Early antimicrobial treatment of dilated cardiomyopathy associated with *Borrelia burgdorferi*

SIR,—Dr Bergler-Klein and colleagues (Aug 1, p 317) report "no important changes in left ventricular ejection fraction" with antibiotic treatment of *Borrelia burgdorferi*-associated dilated cardiomyopathy (DCM). These findings seem to be in contrast to our data (May 9, p 1174) that showed a reversal by ceftriaxone of DCM in 6 of 9 patients and an improvement of left ventricular ejection fraction in 2 patients. Bergler-Klein and co-workers investigated DCM patients who had cardiac dysfunction for up to 25 years before antibiotic treatment (mean about 5 years), whereas mean duration of cardiac dysfunction before enrolment in our study was only 3·7 (SE 0·7) months. In this open, comparative, non-randomised study, the 42 patients were consecutively admitted to our department over 2 years. In the *B burgdorferi* group, the mean duration of cardiac symptoms before treatment was 2·9 (0·7) months. Hence, the two reports refer to different cohorts of patients.

The work of Bergler-Klein et al reveals an important point: the duration of DCM before antibiotic treatment may play a crucial part in the clinical outcome of *B burgdorferi* associated DCM. It is, of course, unlikely that DCM of more than 5 years' duration could be reversed by antibiotic treatment since structural alterations of the myocardium (eg, fibrosis, reduction of myocytes) are predominant, whereas in earlier stages the inflammatory component will prevail. Nor will multiple cycles of antibiotics or switching to other antibiotics, as tried by Bergler-Klein in longlasting DCM, be of much help. The fact that the only substantial clinical improvement was seen in patients with *B burgdorferi* associated DCM for less than 6 months before antibiotic treatment lends support to the idea that time between onset of symptoms and start of treatment is important.

We have examined the relation of time between onset of symptoms and treatment and the improvement of left ventricular ejection fraction in our *B burgdorferi* patients (figure). The



Relation of time between onset of cardiac symptoms and start of treatment and improvement of left ventricular ejection fraction in patients with *B burgdorferi*-associated dilated cardiomyopathy.

correlation between the duration of symptoms and improvement in left ventricular ejection fraction was 0·68 in 9 subjects. Improvement is unlikely after more than 6 months. It is noteworthy that in 5 (15%) of the 33 DCM patients without a history of *B burgdorferi* infection, DCM could be reversed in the early stages by angiotensin-converting enzyme (ACE) inhibitors, diuretics, digitalis, and the patient refraining from alcohol and physical strain. The mean duration of symptoms before treatment in these patients was 3·9 (0·9) months.

These findings are to some extent similar to results of multicentre studies on the effect of ACE inhibitors on left ventricular function after acute myocardial infarction (early onset of treatment).[1,2] That Bergler-Klein et al do not see any improvement in their DCM patients possibly indicates the non-reversibility of structural damage in a group of patients with a long duration of cardiac dysfunction before treatment.

Finally, we apologise for the mistake in authorship of ref 1 in our article,[3] but we believe that we did not cite the reference incorrectly in the context of cultivating *B burgdorferi* from a DCM patient.[4]

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African tick-bite fever: a new spotted fever group rickettsiosis under an old name

SIR,—In the 1930s it was thought that two spotted fever group (SFG) rickettsioses occurred in South Africa.[1] One was boutonneuse fever caused by *Rickettsia conorii* and transmitted by ticks from dogs in urban areas, and the other was tick-bite fever, a milder disease caused by a different SFG rickettsia and transmitted by ticks (*Amblyomma hebraeum*) of cattle and game in rural areas. This idea fell into disfavour and tick-bite fever has become the name of the disease caused by *R conorii* in southern Africa.[2]

In August, 1992, a 36-year-old woman presented to the Chiredzi Consulting Rooms with a history of tick-bite behind the right ear, high temperature, and a severe headache. The skin at the bite was erythematous and she had regional lymphadenopathy but no maculopapular rash. After 3 days of cotrimoxazole and 5 days of erythromycin, the clinical symptoms resolved.

An SFG rickettsia was isolated from heparinised blood collected on the fifth day and analysed by polymerase chain reaction and restriction endonuclease fragment length polymorphism with oligonucleotide primer pairs for the 190 kDa (Rr190·0p and Rr190·602n)[3] and 120 kDa (BG1 and BG2)[4] antigen genes of *R rickettsii*, and *Rsa*1 and *Pst*1 restriction endonucleases. In our laboratory (Unité des Rickettsies) the use of these primer pairs and restriction endonucleases has enabled us to differentiate rapidly between the pathogenic species of SFG rickettsiae. Our isolate was different from *R conorii* and the other pathogenic SFG rickettsiae (*Rickettsii, sibirica, australis, japonica,* and *akari*) and the Israeli and Thai tick typhus rickettsiae. It was, however, identical to six SFG rickettsia isolates from *A hebraeum* collected around Zimbabwe and to an SFG rickettsia isolated from *A cohaerens* in Ethiopia.

The isolation of this pathogenic SFG rickettsia enables us to confirm that there are two SFG rickettsioses in southern Africa. One is boutonneuse fever caused by *R conorii* and transmitted by ticks from dogs, and the other is African tick-bite fever caused by an SFG rickettsia transmitted by *Amblyomma* spp. We propose that the new SFG rickettsia be named "*Rickettsia africae*".

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Pregnancy after induction of ovulation with recombinant human FSH in polycystic ovary syndrome

SIR,—Transfection of human follicle-stimulating hormone (FSH) subunit genes into Chinese hamster ovary cells results in secretion of the intact FSH dimer.[1] Bioactivity and pharmacokinetic properties of recombinant human FSH (rhFSH) are similar to natural FSH in animals[2] and man.[3] Moreover, follicle development can be induced in a hypogonadotropic woman with rhFSH only, and luteinising hormone (LH) is mandatory for adequate oestrogen production.[4] Pregnancies have now been reported after exogenous rhFSH in in-vitro fertilisation programmes.[5,6] Induction of ovulation in patients with polycystic ovary sydrome (PCOS) resistant to clomiphene citrate with human menopausal gonadotropins (hMG) is associated with an increased risk of ovarian hyperstimulation and multiple pregnancies.

A 27-year-old woman (body mass index 25 kg/m²) had been infertile for 3 years due to chronic clomiphene-resistant anovulation. PCOS diagnosis was based on: oligomenorrhoea (cycle length 8–52 weeks), increased serum LH (14·5 IU/l), hyperandrogenaemia (testosterone 5·1 [0·5–3·0] nmol/l, dehydroepiandrosterone sulphate 10·1 [1·2–10] μmol/l), and polycystic appearance of ovaries by transvaginal sonography. Previous induction of ovulation with hMG and adjuvant gonadotropin-releasing hormone agonist resulted in six ovulatory cycles, but no pregnancy was achieved. As part of a multicentre trial (approved by the local ethics committees) rhFSH (Org 32489) was started on the third day of progestagen withdrawal bleeding with daily intramuscular administration of 75 IU. On day 10 ultrasound revealed the development of a single follicle (12 mm diameter). On day 13 the follicle reached 18 mm and 10 000 IU human chorionic gonadotropin (hCG) was given intramuscularly. 2 days later ovulation was confirmed by sonography. No luteal support was given. 16 days after hCG, a pregnancy test was positive and 2 weeks later ultrasound revealed an intact intrauterine pregnancy.

This case shows the capacity of rhFSH to induce monofollicular development and adequate luteal function resulting in a viable pregnancy.

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Simple method for cystic fibrosis carrier screening

SIR,—While cystic fibrosis (CF) carrier screening in the general population is controversial, previous trials have shown widespread acceptability and a positive psychological impact among prospective parents.[1,2] It has been suggested that general screening would be too expensive and inefficient until the method had a high detection rate (85–95%).[3] In several North European countries, this detection rate of couples at risk can be achieved by testing only the most common CF mutation, ΔF508. With a two-step approach, screening primarily the ΔF508 in couples and more mutations only in the partners of ΔF508 carriers, over 85% of couples at risk could be identified in any community in which the ΔF508 mutation is present in more than 65% of the CF chromosomes.[4]

Here we describe a simple and cheap method for population carrier screening of ΔF508 with small pieces of blood spots on filter papers as a template for the allele specific polymerase chain reaction (PCR) amplification[5] and a pooling strategy.[6] 300 health workers of Hospital Niño Jesús without a family history of CF volunteered for the study. A small drop of blood from each individual taken from skin puncture was placed on a Guthrie card under sterile conditions. Filter paper pieces of about 1–2 mm² with dried blood from 20 different individuals were mixed in the same reaction tube containing (100 μl total volume) PCR buffer, 200 μmol/l of each deoxynucleotide triphosphate, and 30 pmol of each primer. The mixture was overlaid with 60 μl paraffin oil and placed in a thermocycler. After a first DNA liberation and denaturation step at 97°C for 8 min, 2·5 U *Taq* polymerase was added per tube during the first annealing step at 64°C for 2 min. After another 2 min at 72°C, 34 additional amplification cycles (94°C for 1 min, 64°C for 45 s, and 72°C for 2 min) followed with a final extension of 10 min at 72°C.

Of the 15 lanes with all the 300 samples run in a single non-denaturing 8% polyacrylamide gel, heteroduplexes could be identified in 3 by staining with ethidium bromide. A subsequent pooling step divided the 60 samples of possible carriers in 6 reaction tubes, and so on.

With a total of 41 reactions, 3 carriers for the ΔF508 mutation were identified in this population. Individual analysis of each of the 300 samples confirmed the 3 ΔF508 heterozygotes and did not show any false-negatives. Although the occurrence of a mutation in an amplification-resistant sample might not be detected by this method, we did not find any. We have not seen contamination problems with Guthrie spots.