hypertrophic cardiomyopathy[1] by DDD pacing. Third, despite the fact that the patients were mainly in New York Heart Association functional class IV before DDD pacing, in both types of cardiomyopathy a considerable increase in exercise capability is achieved.[1,4] Moreover, in both conditions the beneficial effects of chronic DDD pacing continue to be evident when pacing is abruptly stopped.[1,4] During 5 years' follow-up a continuous decrease in the size of the left ventricle was seen in dilated cardiomyopathy.[4] These important findings indicate that in both types of cardiomyopathy DDD pacing in the long run seems to have a beneficial impact on the damaged myocardium, which probably undergoes secondary cellular/molecular changes in response to the altered pattern of electrical activation.[1,4]

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Mitochondrial DNA mutations in the myelodysplastic syndrome

SIR,—Rotig et al[1] have reported the deletion of mitochondrial DNA (mtDNA) in the haematopoietic tissue of a patient with Pearson's syndrome, a fatal bone marrow disorder with clinical features similar to the myelodysplastic syndromes (MDS). This prompted us to examine patients with primary MDS for mtDNA mutations. The molecular analysis of mtDNA is usually difficult and requires complex methodologies such as DNA sequencing. Thermal-gradient-gel electrophoresis (TGGE)[2,3] is a rapid alternative to screen for base mutations in specific regions of mtDNA.

We extracted high molecular weight DNA from a marrow aspirate taken from a 57-year-old white man at the time MDS was diagnosed (refractory anaemia FAB subtype) and again 16 months later when he presented with acute myeloid leukaemia (AML). Standard sequence DNA was obtained from the peripheral blood of a normal white man and in-vitro polymerase chain reaction testing was done on all samples[4] with primers spanning position 5'-14724 (heavy strand) to 15149-3' (light strand) of human mtDNA. This delineated PCR fragment encompassed some 498 bp of the cytochrome b gene. PCR product cross-hybridisation[5] was followed by TGGE analysis of the products.[1]

We observed a single, polymorphic melting domain with respect to the standard in the cytochrome b gene of mtDNA isolated at the time of diagnosis of MDS. Heteroduplex fragment denaturation began at 39°C with complete strand separation at 52°C. Repeat TGGE 16 months later showed a second additional sequence polymorphism in the cytochrome b gene with heteroduplex denaturation starting at 37°C and strand separation at 51°C. The calculated degree of difference between the patients' samples with respect to the standard[6] indicated that mutation of the second melting domain occurred as the patient progressed from MDS to AML.

The importance of mtDNA mutations in the pathogenesis of MDS cannot be assessed until more patients have been analysed. However, the importance of normal mtDNA in haematopoiesis is illustrated by the haematological abnormalities that result from clonal deletions of mtDNA in the bone marrow (Pearson's syndrome)[6] and the suppression of mitochondrial protein synthesis by chloramphenicol.[7] Similar haematological defects occur in patients with MDS. This suggests a causal relation between the clinical features of this disorder and mtDNA in bone marrow. TGGE is a practical method to screen patients suspected of genetic mutation in defined regions of the mitochondrial genome.

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Prognostic value of serum β_2-microglobulin in HIV infection

SIR,—Dr Lifson and colleagues (June 13, p 1436) describe the importance of serum β_2-microglobulin concentrations as predictors of the progression to AIDS in people infected with HIV, indicating that monitoring of β_2-microglobulin might have therapeutic implications. We evaluated β_2-microglobulin in HIV-infected intravenous drug users (IVDU) undergoing long-term treatment with zidovudine.

β_2-microglobulin (Latex immunoassay, normal range 1·2–2·5 mg/l) concentrations were measured in 36 HIV-infected patients with stage II disease (CDC, Atlanta), treated with zidovudine 500 mg/day and 36 patients with AIDS receiving 750 mg/day. β_2-microglobulin was measured at 3, 6, 9, and 12 months of treatment and compared with CD4 lymphocyte counts:

	Months				
	0	3	6	9	12
*β_2-microglobulin (mg/l)**					
HIV infection†	2·7 (0·8)	2·3 (0·6)	2·5 (0·6)	2·7 (0·7)	2·7 (0·6)
AIDS	3·1 (1·0)	3·0 (1·0)	2·8 (1·1)	3·0 (0·5)	3·1 (0·3)
*CD4 counts (/μl)**					
HIV infection	204(174)	213(131)	258(213)	145(183)	192(170)
AIDS	98(129)	87(107)	98(198)	79(121)	111(165)

*Means (SD) shown, †including 4 patients with stage II disease who went on to develop AIDS

In patients with stage II infection, β_2-microglobulin dropped significantly at 3 months and 6 months ($p < 0·05$); at the same time CD4 counts rose significantly ($p < 0·05$). After 9 months of treatment β_2-microglobulin stabilised. A similar drop in β_2-microglobulin at 9 months (from 3·2 [0·6] to 2·7 [0·7] mg/l $p < 0·01$) was seen in 4 of these patients who developed AIDS. CD4 counts in these 4 patients rose significantly up to 6 months, (from 64 [61] to 169 [164]/μl at 6 months; $p < 0·01$).

In the patients with AIDS serum β_2-microglobulin fell significantly ($p < 0·05$) at 6 months, rising again at 9 months. CD4 counts were stable. 14 of the 36 patients died or had secondary opportunistic diseases. In these 14, β_2-microglobulin dropped from 3·5 (0·5) to 3·1 (1·6) ($p < 0·05$) at 6 months, with stable CD4 counts.

These results show that serum β_2-microglobulin concentrations fall in HIV-infected patients receiving zidovudine, as Lifson et al and others have reported.[1-3] Concentrations fell progressively up to 6–9 months after start of treatment in both groups of patients, whereas CD4 counts rose, during the same period, only in the patients with CDC stage II disease. However, outlook with respect

to survival for HIV-infected patients, as found by Jacobson et al,[4] could not be judged from β_2-microglobulin values since there were no differences in concentrations between HIV-infected patients, those who developed AIDS during the study, those who died, and those who presented with secondary opportunistic diseases.

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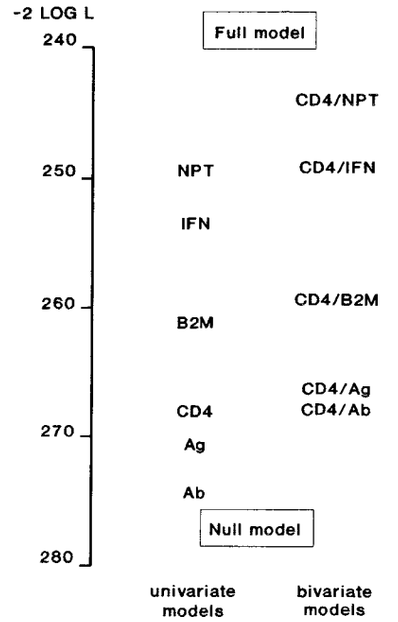
SIR,—Evaluation of 214 HIV-1 seropositive homosexual men by Dr Lifson and colleagues (June 13, p 1436) provides additional evidence that measurement of β2-microglobulin adds information to CD4 counts for estimating the risk of AIDS. This result accords with and extends analyses of immune activation markers such as β2-microglobulin and neopterin as predictors of AIDS.[1-5]

We extended our investigation of a cohort of 131 homosexual men with incident HIV-1 infection to include various immunological and virological markers.[6] In addition to CD4 counts, β2-microglobulin, and p24 antigen, we evaluated serum concentrations of interferon, neopterin, and p24 antibodies. On the basis of measurements at various year points—eg, three years after seroconversion—all markers apart from anti-p24 were significant predictors of AIDS risk in univariate analyses. Compared with the null Cox regression model, neopterin and interferon concentrations were the best single predictors, followed by β2-microglobulin concentrations and CD4 counts (figure). Bivariate analyses revealed a similar picture, showing neopterin, interferon, and β2-microglobulin as significant joint predictors with CD4%. Even more information was obtained by including the three best markers neopterin, interferon, and CD4%. β2-microglobulin was strongly correlated with neopterin, and seemed to be a better predictor of AIDS later rather than earlier in the course of disease (eg, five years after seroconversion). In addition, the value of these markers may vary with the different manifestations of AIDS, since interferon strongly predicted opportunistic infections but not Kaposi's sarcoma.[6]



Predictive value for developing AIDS of immunological and virological markers measured three years after HIV-1 seroconversion.

For comparison of predictive values, −2 log likelihood estimates are shown for single markers and for marker combinations The −2 log likelihood estimate for the "Null model" (277·16) was improved in the "Full model" (238·45) with all six markers. NPT = neopterin, IFN = interferon, B2M = β2-microglobulin, CD4 = CD4%, Ag = p24 antigen, Ab = anti-p24 antibody. Neopterin and CD4 plus neopterin were the best univariate and bivariate models, respectively

Our results generally accord with the conclusions of Lifson and co-workers and contribute to the evidence that measurement of serum soluble immune activation markers improves the prediction of AIDS risk in individuals with HIV infection. Our data also support the view that immune activation is involved in the pathogenesis of AIDS.

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SIR,—Serum concentrations of β_2-microglobulin are used prognostically to monitor and predict progression of HIV infection and, as Dr Lifson and colleagues report, they have a good correlation with progression of the disease in homosexual and bisexual men. Other published studies have evaluated its use as a prognostic marker in that population, and in recipients of blood products.[1,2] However, there is some evidence that drug-injecting behaviour can raise serum β_2-microglobulin, and thus its prognostic reliability in HIV-infected injecting drug users (IDUs) may be affected.[3,4]

We are conducting a prospective study to assess the prognostic value of serum β_2-microglobulin concentrations and other surrogate markers in a cohort of 327 patients with HIV infection followed for a mean of 4·7 months. Serum β_2-microglobulin concentrations were measured with a quantitative radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden) at 3–6-month intervals. Statistical analyses were done by means of the Statview II statistical package (Abacus Concepts, Berkeley, California, USA). 226 patients were drug users (69%), 52 were homosexual or bisexual men (16%), 45 were non-drug-user heterosexual men or women (14%), 3 were recipients of blood products (0·9%), and 1 (0·3%) had unknown risk factors for HIV infection. The table shows baseline serum β_2-microglobulin concentrations according to risk factor and Center for Disease Control (CDC) staging. Serum β_2-microglobulin concentrations did not differ between the groups with respect to risk factor for HIV infection, once CDC staging of the disease was taken into account. Reliable information about their drug-injecting behaviour could be assessed in 125 (55%) drug users. We classified drug users as non-injecting (non-IDU) when history, clinical signs, and urine toxicology analysis did not suggest injecting drug use for at least one month before baseline β_2-microglobulin measurement. 97 of 125 drug users (78%) were non-IDUs, whereas 28 (22%) were IDUs. Mean serum β_2-microglobulin concentrations at

BASELINE SERUM β_2-MICROGLOBULIN (mg/l) CONCENTRATIONS IN 327 PATIENTS WITH HIV INFECTION*

CDC staging	Risk behaviour: IDUs† (n=226)	Homosexual/ bisexual men‡ (n=52)	Non-IDU, heterosexuals§ (n=45)
II	3·55 (1·08)	3·16 (1·00)	3·12 (1·2)
III	3·89 (0·97)	4·73 (1·46)	3·31 (0·67)
IV	4·07 (1·12)	4·30 (1·12)	4·08 (1·20)

*Means (SDs) shown. †II *vs* III p=0·038; II *vs* IV p=0 001, III *vs* IV p=0 49 (not significant). ‡II *vs* III p=0·008; II *vs* IV p=0·0008; III *vs* IV p=0 23 (NS). § II *vs* III p=0·74; II *vs* IV p=0·04; III *vs* IV p<0·21.

baseline were 4·07 (SD 1·03) mg/l for IDUs, 3·63 (1·08) mg/l for non-IDUs (p=0·058), 3·63 (1·20) mg/l for homosexual or bisexual men (p=0·11), and 3·35 (1·20) mg/l for heterosexual patients (p=0·01) (p values are all for comparison with IDU group). The groups did not differ according to CDC staging of HIV infection. Additionally, serum β_2-microglobulin concentration correlated positively with the duration of drug addiction ($r=0·24$, p=0·02), but no correlation was found between β_2-microglobulin concentration and time free of drug-injecting behaviour ($r=0·07$, p=0·45). β_2-microglobulin concentration was significantly and negatively correlated with CD4 count ($r=-0·29$, p=0·0011) for both IDUs ($r=-0·41$, p=0·03) and non-IDUs ($r=-0·29$, p=0·0047). Furthermore, β_2-microglobulin correlated positively with other surrogate markers such as neopterin ($r=0·71$, p=0·0001) and IgA ($r=0·31$, p=0·0006).

β_2-microglobulin is produced by stimulated lymphocytes, and thus its serum concentration reflects the degree of immune-system activation.[5] Our results suggest that changes in β_2-microglobulin in HIV-infected drug users indicate differences in drug-injecting behaviour. However, even in actively injecting drug users β_2-microglobulin concentrations are positively correlated with CD4 cell count, and this suggests that β_2-microglobulin can also be a useful prognostic marker in HIV-infected drug users. The positive correlation with serum neopterin, a good prognostic marker in drug users[4] lends further support to its usefulness.

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TNFα in stool as marker of intestinal inflammation

SIR,—Dr Braegger et al[1] suggested that tumour necrosis factor alpha (TNFα) concentrations in stool provide a simple way to monitor disease activity in inflammatory bowel disease. They also measured TNFα in stools of 14 children with acute diarrhoea, of whom 3 had concentrations above the control range. They suggested that stool TNFα may be raised in infectious colitis. We have measured TNFα and interleukin-6 by ELISA in stool filtrates of children with acute diarrhoea[2] (table).

Our study confirms that in children with shigella diarrhoea, TNFα concentrations are in the range reported for children with

TNFα AND INTERLEUKIN-6 CONCENTRATIONS IN STOOL OF CHILDREN

—	n	TNFα (pg/ml)	Interleukin-6 (pg/ml)
Shigella dysenteriae 1	13	7 (12–2545)*	8 (20–8044)
S flexneri	9	3 (25–358)	3 (12–68)†
Non-typhoid salmonellae	5	ND	1 (6)
Cryptosporidia	5	ND	ND
Rotavirus	4	ND	ND
Adenovirus	4	2 (10, 109)	ND
Pathogen-negative diarrhoea	4	ND	ND
Without diarrhoea	4	ND	ND

*No of cases with detectable cytokine (range or result)
†n=8.
ND=not detectable; threshold 10 pg/ml for TNFα and 4 pg/ml for interleukin-6

inflammatory bowel disease. We hypothesise that local production of cytokines and other mediators of the inflammatory response may mediate the local and generalised vasculitis that occurs in shigellosis.[3] These data confirm the suggestion that stool TNFα is raised in infectious colitis. In other infective conditions, serum interleukin-6 is a better indicator of severity than TNFα.[4,5] It would be of interest to determine whether the same relation existed in chronic inflammatory bowel disease.

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Tumour metastasis

SIR,—Dr Hart and Dr Saini (June 13, p 1453) review the biology of tumour metastasis. We appreciate the difficulties inherent in condensing such a complex subject to a form that is comprehensible and interesting to the non-specialist, but there are two aspects that I think cannot be over-emphasised. First, much of what is known about the metastatic process (eg, figure 2) is only uncertain hypothesis, having been deduced from models of invasion or metastasis. Many of these models are of questionable validity; certainly, extrapolation from such models to progression, invasion, and metastasis in patients should be done with much caution. For example, the suggestion that cadherins function as suppressors of invasion[1] is based largely on measurements of the invasion by tumour cells into fragments of embryonic chick heart or deposits of collagen in tissue culture dishes.

Second, the discrimination between the metastatic and tumorigenic phenotypes needs fuller consideration. We anticipate that an event that reduces tumorigenicity will inevitably compromise metastatic propensity, yet most studies of metastasis pay scant attention to this connection. Thus, for example, the conclusion that *nm23* is a metastasis suppressor gene[2] ignores the