Authors’ reply

Sir—Although Koster and colleagues1 reported that APC response is not significantly affected by subsequent cycles of freezing and thawing and all published results on APC response have been obtained on frozen samples, aPTT results (which are at the basis of APC response measurement) are undoubtedly affected by freezing, as Girolami and colleagues point out. Their comments give us an opportunity to clarify some aspects that, for the sake of brevity, were not discussed in our previous report on APC in patients with juvenile venous thrombosis (JVT).2

First, all tests in our study—in patients (at first and second control), normal pool, and healthy subjects—were done on frozen samples. We considered the fact that the duration of storage might have been a possible cause of variability in results. The mean storage time of samples used for first screening was significantly longer than that of samples used for the second control (10.5 vs 3.1 months, p<0.001). However, the storage time was not different in samples of patients with confirmed or unconfirmed altered APC response (12.8 vs 8.2 months). Moreover, aPTT ratios (frozen samples vs frozen normal pool) did not differ in the first and second controls (mean 1.0 vs 1.05), nor did they in patients with confirmed or unconfirmed altered APC response. Thus, the observations of Girolami and co-workers though important and germane, do not seem to explain our inconsistent results.

We have no definite explanation for the high variability recorded. The possibility of acquired and transient APC resistance cannot be ruled out. However, on the basis of some of our recent work, it now seems that assay reproducibility is less consistent than we reported. By using more kits (APC Resistance, Chromogenix, Sweden) we have recorded an inter-batch co-efficient of variation (CV) of 17%, which is much higher than the 8% inter-assay CV previously measured. Since handling of samples and instrumentation used (ACL 300 Research) were carefully controlled, we surmise that differences in activity of APC reagent or instability of aPTT reagents in the kits, or both, might substantially affect the reproducibility of results. We believe that the expression of results as a ratio of the values obtained in samples and in normal pools for the same testing session (as originally proposed by the manufacturer), should be adopted to improve assay reproducibility, at least until the reagents are better standardised.

We have now increased the number of patients in our JVT series (n=261) and we can confirm our previous results. We agree with Girolami that the prevalence of APC resistance in these patients, on the basis of only the cases confirmed at resampling, seems to be around 10%. Since in other studies the prevalence of APC resistance varied from about 20% to 65%, we are surprised at the low prevalence in our patients and wonder whether methodological problems may be at least partly responsible for the wide variation between studies.

Cristina Legnani, Guaitiero Palareti, Roberta Biagi, Sergio Coccheri

Department of Angiology and Blood Coagulation, University Hospital S Orsola, 40138 Bologna, Italy


SIR—Our group reported (Koster and colleagues, Dec 18/25, p 1503) that a poor anticoagulant response to APC is a common and strong risk factor for venous thrombosis. This APC resistance becomes apparent by insufficient prolongation of the clotting time (aPTT) when APC is added to patient plasma.1 We set a reference value for the APC-sensitivity ratio (ACP-SR, ratio of aPTT after addition of APC to aPTT in the absence of APC) from results in 301 healthy individuals (mean = 2 SD=2.17). In 301 consecutive patients with a first, objectively confirmed episode of deep venous thrombosis, we found APC resistance in 21% (64 cases). In 301 age-matched and sex-matched healthy controls, APC resistance was present in 5% (14 cases): this yielded an odds ratio for thrombosis associated with APC resistance of 66. Our cut-off points were strictly statistical, which leaves some uncertainty about the diagnosis in individuals. Moreover, Legnani and colleagues describe poor reproducibility of an abnormal ACP-SR. Our finding of that APC resistance is associated with a mutation in the factor V gene (FV-Leiden) at the site where APC cleaves and inactivates clotting factor V, made it possible to re-examine the previously diagnosed cases directly by DNA analysis. Mutant and normal factor V alleles were discriminated with M bel I digestion of an amplified factor V DNA fragment and visualisation of the cleavage products on ethidium bromide-stained agarose gels.3 In controls with normal ACP-SRs—ie, greater than 2.17—we invariably find normal results with this technique.

We present the results of the DNA analysis for all individuals (patients and controls) who had an abnormal ACP-SR (aPTT ratio <2.17) in our initial analysis (this figure is the lower part of our original figure). Of 64 patients with ACP-SR less than 2.17, 53 carried the mutation. 6 of these were homozygous for the mutation. Among the 14 controls with an ACP-SR less than 2.17, 9 carried the mutation. The distribution of individuals with normal and abnormal results in DNA analysis, shows that the cut-off point should be set at an ACP-SR of about 1.90 rather than
with flexor plantar responses. There was symmetrical distal weakness, normal lower limb power, and complete areflexia.

Elevation with reduced gag reflex, impaired cough, and gaze, bilateral Horner's syndrome, absent voluntary palatal ophthalmoparesis with diplopia on left lateral and vertical was alert and fully oriented. The main findings were a mild infection two weeks before admission. His medical, family, and social history were unremarkable. On examination, he had been feeling generally unwell since an upper respiratory tract unsteadiness, and tingling in his hands and feet. He had Miller-Fisher syndrome in three patients. We describe another case.

Figure: DNA analysis of APC resistance
Analysis of 64 patients and 14 healthy controls who were APC resistant (APC-SR <2.17). Open symbols indicate individuals not carrying factor V Leiden mutation, semi-closed symbols individuals who were heterozygous, and closed symbols individuals who proved homozygous for this defect.

at 2.17, as we suggested in our report because of the bimodal distribution of ACP-SRs, with a gap between 1.8 and 2.0. It is noteworthy that there is no overlap of APC-SR values of individuals with and without the factor V mutation.

These results show that in all patients with ACP-SR less than 1.9, the diagnosis of ACP-resistance can be confirmed by DNA analysis. It also shows that, by contrast with Legnani's experience, in our laboratory the clotting test with the ratio of two APTTs performs perfectly.

F R Rosendaal, R M Bertina, P H Reitsma
Departments of Clinical Epidemiology and the Haemostasis and Thrombosis Research Centre, University Hospital Leiden, PO Box 9600, 2300 Leiden, Netherlands


Miller-Fisher syndrome with rapid recovery
Sir—Roberts and colleagues (Feb 19, p 454) report a strong association between GQ1b-ganglioside antibodies and the Miller-Fisher syndrome in three patients. We describe another case.

A 65-year-old retired farmer presented with a one-week history of an altered nasal voice, increasing dysphagia, unsteadiness, and tingling in his hands and feet. He had been feeling generally unwell since an upper respiratory tract infection two weeks before admission. His medical, family, and social history were unremarkable. On examination, he was alert and fully oriented. The main findings were a mild ophthalmoparesis with diplopia on left lateral and vertical gaze, bilateral Horner's syndrome, absent voluntary palatal elevation with reduced gag reflex, impaired cough, and severely impaired swallowing. He had slight upper limb weakness, normal lower limb power, and complete areflexia with flexor plantar responses. There was symmetrical distal impairment of pain perception and proprioception in all four limbs, and he could hardly walk because of striking sensory ataxia.

Routine blood tests were normal. Cerebral spinal fluid analysis showed a slightly raised protein concentration of 0.57 g/L but no cells. GQ1b-ganglioside antibodies were present with a titre of 200. Nerve conduction studies revealed evidence typical of a widespread acute inflammatory polyradiculoneuropathy with unrecordable sensory nerve action potentials in the upper and lower limbs, delayed F waves, and evidence of motor conduction block.

We managed his bulbar palsy with intravenous fluids and soft diet and monitored his pulmonary function with spirometry and peak flow; he had physiotherapy for his mobility difficulties. He recovered rapidly and was discharged three weeks after the onset of symptoms.

This case adds further evidence for the strong association between the Miller-Fisher syndrome and GQ1b-ganglioside antibodies (Roberts and co-workers and refs 1 and 2). The wide spectrum of clinical features in the Miller-Fisher syndrome has been emphasised in a recent review,1 and bulbar presentation is uncommon. Our patient did not complain of diplopia, and the diagnosis may easily be overlooked. Furthermore, most published cases have striking neurological impairments, and mean recovery time is over ten weeks.2 We suspect that milder cases with atypical presentation and rapid recovery such as in this man are underreported.

Alexander Hammers, Richard J Hardie
Department of Neurology, Royal Devon and Exeter Hospital (Wonford), Exeter EX2 50W, UK

Proliferating cell nuclear antigen as a marker of hepatocyte proliferation
Sir—Fang et al (April 2, p 820) report on the use of the monoclonal antibody PC10 against proliferating cell nuclear antigen (PCNA) for the assessment of hepatocyte proliferation in acute alcoholic hepatitis. They imply a good correlation of PCNA staining with another proliferation marker, human histone 3. In 11 of 25 cases, PCNA expression was seen in 31–80% of hepatocytes. However, PCNA has limitations as a marker for hepatocyte proliferation.

We have compared the expression of PCNA with the proliferation-associated Ki-67 antigen in liver biopsies containing metastatic tumour or showing hepatitis, including alcoholic hepatitis.1 Ki-67 is a reliable immunohistochemical marker of proliferating cells.2 We found that PCNA can be expressed by non-proliferating hepatocytes—indeed, it may be expressed in large numbers of hepatocytes in histologically normal liver. In alcoholic hepatitis, we found that there was generally high expression of PCNA in hepatocyte nuclei compared with low expression of Ki-67 antigen. Furthermore, PCNA is overexpressed by hepatocytes in post-transplant liver biopsies showing acute rejection (unpublished). We postulate that cytokines or other growth factors released from nearby inflammatory cells...