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Letters to the Editor

Gamma irradiated blood samples: unsuitability for haemostatic evaluation of high risk patients

Over the past 40 years, several "new" viruses have been discovered which cause haemorrhagic and often fatal disease in man; Lassa, Ebola, and Marburg are but three examples. The haemostatic defect(s) in these diseases remain(s) largely undefined, partly because blood samples from infected patients can only be handled by centres equipped with P-4 containment facilities.[1] To determine the feasibility of using inactivated viral specimens for haemostatic analysis we studied the effects of gamma irradiation on several laboratory coagulation variables.

For plasma assays, one unit of time-expired, fresh frozen plasma was thawed at 37° C, and 0·5 ml portions were aliquoted into sets of 1·8 ml cryule tubes, six tubes to each set. For serum assays, 10 ml of fresh blood from a healthy donor were placed in a glass tube in a water bath at 37° C; after one hour the middle portion of the serum was divided equally into a further set of six cryule tubes. Three tubes from each set were exposed to 4×10^6 rads of gamma radiation using a gamma cell containing ^{60}Co (model 220; Atomic Energy of Canada Ltd, Ottawa, Canada) and three were left unirradiated; during the procedure, all test and control samples were kept at below 4° C in dry ice. Specimens were stored at −70° C until analysed.

We measured prothrombin times (PT), partial thromboplastin times (PTTK), and thrombin times (TT) manually by standard techniques; fibrinogen concentrations by the Clauss method; factor VIII by a standard one-stage assay; von Willebrand factor antigen (vWFAg) by an ELISA method modified from that of Short *et al*; ristocetin cofactor (RiCof) by platelet aggregometry using washed platelets[2]; antithrombin III (ATIII) by a microtitre amidolytic assay modified from that of Odeggard *et al*[3]; protein C by a microtitre amidolytic (snake venom activated) assay[4] using British Standard (NIBSC, Hertfordshire); protein S by an ELISA technique using polyclonal antiserum (Dakopatts, Buckinghamshire) and rabbit anti-human peroxidase conjugate (concentrations were standardised using pooled plasma from 20 donors); and serum thromboxane B_2 (TXB_2) by radioimmunoassay (Amersham, Buckinghamshire).

With the exception of vWFAg, all these tests were considerably affected by gamma irradiation (table). Thrombin times, PTS, and PTTKs were all prolonged in irradiated samples compared with control samples, and there was generally considerable variability in the extent of prolongation in replicate aliquots. Concentrations of fibrinogen, factor VIII, RiCof, antithrombin III and protein C, which were all obtained by functional assays, showed varying degrees of reduction after gamma irradiation. Concentrations of serum TXB_2 and protein S, although determined by immunological methods, were similarly affected.

These data show that gamma irradiation at a dose sufficient to inactivate class IV viral agents[5] profoundly affects most haemostatic variables in serum and plasma. No method of viral inactivation has yet been described which does not affect the concentrations of coagulation factors in human blood. Samples obtained from patients suspected of harbouring a class IV pathogen must therefore be analysed in a maximum containment laboratory.

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Table *Effect of gamma radiation on laboratory coagulation assays*

Laboratory test	*Irradiated*	*Non-irradiated*
PT (s)	20, 25, 52	18, 20, 21
PTTK (s)	51, 56, 135	38, 41, 38
Thrombin time (s)	20, 19, 19	13, 12, 12
Fibrinogen (g/l)	<0·10, 0·84, 0·50	2·10, 2.20, 2.20
Factor VIII (IU/ml)	0·14, 0·24, 0·11	0·48, 0·46, 0·50
vWFAg (IU/ml)	0·50, 0·48, 0·56	0·48, 0·52, 0·52
RiCof (IU/ml)	<0·05, <0·05, 0·11	0·72, 0·72, 0·72
ATIII (IU/ml)	0·27, 0·52, 0·37	0·94, 0·95, 1·08
TXB_2 (ng/ml)	180, 85, 264	327, 314, 330
Protein C (IU/ml)	0·12, 0·21, 0·16	0·76, 0·78, 0·80
Protein S (%)	32, 11, 72	88, 89, 86

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Idiopathic haemochromatosis in north Portugal: association with haplotype A3B7

The association of certain HLA antigens and haplotypes with idiopathic haemochromatosis is well documented, and their pattern of distribution has been defined in several countries. The antigen most strongly associated with idiopathic haemochromatosis is HLA-A3 and the haplotypes more frequently linked to the disease are A3B7 and A3B14.[1-3]

In 1986 we started a systematic search for cases of idiopathic haemochromatosis in the north of Portugal that has led to the identification of 30 patients to date. Preliminary studies of the first unrelated patients and families[4] indicated an association of antigen A3 and haplotype A3B7 with the disease in this region.

Seventeen unrelated, HLA typed, patients with idiopathic haemochromatosis (15 men and two women), aged between 21 and 69 years were included in this study. Haemochromatosis was diagnosed according to previously established clinical, biochemical, and histopathological criteria.[5]

One hundred and eighteen family members (55 men and 63 women) of 10 unrelated subjects were studied. All subjects were HLA typed; evaluation of their iron state was carried out by routine measurements of serum iron, total iron binding capacity, transferrin saturation and serum ferritin. In

Table *Prevalence of phenotypes and haplotypes in patients and controls*

	Patients (n = 17)	*Controls (n = 203)*	*Relative risk*
*HLA antigens**			
A2	0·471	0·419	ns
A3	0·529	0·217	4·065 a
A9	0·294	0·222	ns
B7	0·353	0·089	5·606 b
B12	0·353	0·315	ns
B35	0·235	0·108	ns
*HLA haplotype**			
	(16 haplotypes)	(110 haplotypes)	
A3B7	0·176	0·018	12·46 c
A9B5	0·118	0·027	ns
A2B12	0·118	0·118	ns

a $\chi^2 = 7{\cdot}419$ $p < 0{\cdot}01$
b $\chi^2 = 9{\cdot}329$ $p < 0{\cdot}01$
c $\chi^2 = 6{\cdot}920$ $p < 0{\cdot}01$
*only the most common antigens and haplotypes are represented.

the course of the family screening seven additional patients were identified in five of the families. To calculate the haplotype prevalence we followed the criteria defined by Simon *et al.*[1]

The phenotype prevalence of the HLA antigens in the control population was determined in 203 healthy, unrelated blood donors from the blood bank of the Hospital Geral de Santo António-Porto.

HLA haplotype prevalence in the control population was calculated in a control group of unrelated subjects from the north of Portugal, including the spouses of some family members in whom the disease was excluded, heterozygous family members (only the non-idiopathic haemochromatosis associated haplotype was considered), and normal subjects previously HLA genotyped.

The significance of the differences observed between the prevalence of antigens and haplotypes in the patient and control groups was determined by the χ^2 test. The relative risk was calculated by the method of Woolf.

The comparison of the phenotype prevalences of HLA antigens showed a significantly higher prevalence of the A3 and B7 ($p < 0{\cdot}01$) in the patient group. The antigen A3 was represented in 52·9% of the subjects in the patient group and in 21·7% of the controls. The antigen B7 was represented in 35·3% of the cases in the patient group and in 8·9% of the controls. These figures correspond to relative risks of 4·065 and 5·606 for A3 and B7, respectively. Among the other antigens tested, high prevalences of A2 and B12 were also found, but they were seen as often in the control group.

Sixteen haplotypes were associated with the disease in the 10 families studied. In two subjects it was not possible to identify the haplotypes. A3B7 was the haplotype found most frequently in the patient group (17·6%). This was significantly higher than that observed in the control population (1·8%), representing a relative risk of 12·46. The haplotype A3B14, which has also been shown in association with idiopathic haemochromatosis in certain regions,[1] was not found to be linked to the disease in this patient population; this haplotype, however, was not represented in the control population studied.

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Angioimmunoblastic lymphadenopathy associated with thyroid disease

Between 1979 and 1983, nine patients with angioimmunoblastic lymphadenopathy were seen in Norwich Health District. Of these, two patients were found to have associated thyroid disease.

Case 1

A 74 year old widow was admitted because of lack of energy for four weeks follow a "flu-like" illness and a rash on the arms and legs. She had been well, but was receiving fenoprofen 600 mg three times a day for cervical spondylosis.

On examination she was pale and ill, with widespread lymphadenopathy and hepatosplenomegaly. Investigations showed mild normocytic normochromic anaemia, a normal erythrocyte sedimentation rate, and white cell counts; a direct Coomb's test was positive and reticulocyte count was 2%. Serum protein electrophoresis showed a polyclonal increase in gamma globulins, but otherwise serum biochemistry was normal. An infraclavicular lymph node biopsy specimen showed features of angioimmunoblastic lymphadenopathy.

She was treated with prednisolone 10 mg four times a day, but in spite of this she became increasingly anaemic and required transfusions. Her condition rapidly deteriorated and she died of uncontrolled disease and haemolytic anaemia three weeks after admission.

Necropsy showed widespread generalised lymphadenopathy and an enlarged liver and spleen. The thyroid gland (30 g) was firm and showed a nodular, greyish cut surface, suggesting either Hashimoto's disease or tumour infiltration.

Histological examination of the thyroid gland showed follicular atrophy and lymphocytic and plasma cell infiltration consistent with advanced Hashimoto's disease. Lymph nodes were examined from the mesentery hilar regions of lungs, tonsils, and inguinal regions. They had shown the former appearance of angioimmunoblastic lymphadenopathy but with terminal immunoblastic transformation. Immunoperoxidase preparation of necropsy tissue were unsatisfactory as it was not possible to determine if there was a T or B cell preponderance.

Necropsy showed regression of the lymphadenopathy, an osteitis fibrosa-like appearance of vertebrae, and metastatic calcification of blood vessels and alveolar walls. The parathyroids were normal. The thyroid showed residual papillary carcinoma confined to the gland. There was no histological evidence of thyroiditis and no macroscopic evidence of generalised lymphoma.

Case 2

A previously fit 75 year old housewife was admitted with bilateral swelling of the ankles and a generalised purpuric rash of three