features, T-cell intracellular antigen (TIA) expression and p16 and/or p15 methylation status. ALK, TIA and the International Prognostic Index (IPI) were also evaluated as outcome predictors in the ALCL patients.

ALK-1, and anti-NPM antibodies were used for the immunohistological study. RNA was isolated from 21 cases with available frozen material (six ALCL, two DLBCL, one PTCL and 12 HL) and NPM-ALK chimaeric transcripts were detected by nested reverse-transcription polymerase chain reaction (RT-PCR). The methylation status of p15INK4b and p16INK4a genes was determined in 27 ALCL by methylation-specific PCR (MSP) (Herman *et al*, 1996). The method of Kaplan and Meier, and the log-rank test were used for the survival curves. A multivariate survival model was performed using logistic regression analysis.

Only ALCL patients expressed ALK protein (41%) or were positive in the molecular study. Interestingly, one positive sample in the nested-PCR second round of amplification did not show ALK staining. As shown in Table I, ALK^+ ALCL patients were significantly younger, had shorter evolution time prior to diagnosis, presented a higher incidence of bulky disease and more frequently showed TIA-1 expression than the ALK^- ALCL patients. p16/p15 hypermethylation was less frequently observed in the ALK^+ ALCL than in the ALK^- ALCL. The 5-year overall survivals of ALK^+ and ALK^- patients were comparable, the Kaplan–Meier survival curves being similar ($P = 0{\cdot}778$). Cytotoxic phenotype was not associated with prognosis, but IPI was found to be prognostically important. In the multivariate analysis, including sex, age, ALK expression and IPI, only IPI score was significantly associated with mortality [adjusted Odds Ratio (OR) = 4·7].

Our results support that ALK rearrangements are specific markers for T-null ALCL. Many of the 'unspecific' findings reported in the literature may be due to misdiagnosis or to the use of very sensitive techniques that yield false-positive results, which seemed to be the case in our sample that showed discrepancies between the immunohistochemical and molecular analyses. A high percentage of ALCL patients expressed TIA protein (68%), adding force to the hypothesis that a cytotoxic-activated cell is the normal counterpart for nearly all these lymphomas.

Differences were not found in the 5-year overall survival of the ALK^+ and the ALK^- ALCL, probably as a result of the small size of this series, but our data provide evidence that IPI is able to predict outcome in ALCL. Both p16 and p15 methylation frequencies were lower in the ALK^+ than in the ALK^- cases; this may contribute to the better outcome reported for the ALK^+ patients. More studies are needed to assess whether these epigenetic anomalies are prognostic markers independent of ALK expression in ALCL.

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Keywords: ALCL, ALK, p16INK4a/p15INK4b methylation, TIA, outcome.

THE SUCCESSFUL USE OF PLASMA EXCHANGE AND IMMUNOSUPPRESSION IN THE MANAGEMENT OF ACQUIRED GLANZMANN'S THROMBASTHENIA

We describe a patient with acquired Glanzmann's thrombasthenia who developed a life-threatening gastrointestinal haemorrhage. A combination of corticosteroids, azathioprine and plasma exchanges led to normalization of platelet function and cessation of gastrointestinal bleeding. The management was further complicated by the development of heparin-induced thrombocytopenia associated with ileofemoral thrombosis. This was successfully treated by mechanical embolectomy followed by anticoagulation with hirudin.

A 60-year-old woman presented with a 2 month history of easy bruising. There was no past or family history of

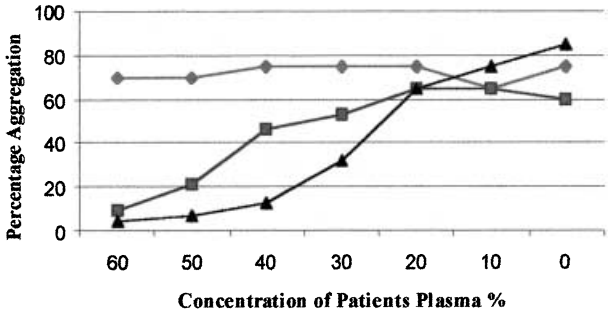

Fig 1. Progressive improvement in aggregation of normal platelets with increasing dilutions of patient's plasma. Platelet-rich plasma = 150×10^9/l. Incubation for 15 min. Ristocetin 1·5 μmol/l, ◆; collagen, ■; ADP 2 μmol/l, ▲.

bleeding. Clinical examination was normal. Investigation revealed a near normal full blood count (haemoglobin 10·6 g/dl, white cell count 6×10^9/l with normal differential counts, platelet count 321×10^9/l), and normal renal and liver function tests. Salicyclates were not detected in urine. The activated partial thromboplastin time (APTT), prothrombin time, fibrinogen, individual coagulation factors and von Willebrand factor were normal. Her bleeding time was prolonged at > 15 min.

Platelet aggregation tests revealed no aggregation with 2 μmol/l and 5 μmol/l ADP, 2 μmol/l and 5 μmol/l adrenaline, or with arachidonic acid, minimal aggregation to collagen (8%), weak responses to U46619 0·3 μmol/l and 1·0 μmol/l (25% and 21%), and normal response to ristocetin (85%) (PAP 4D; Alpha Laboratories, UK). Platelet nucleotides and 5-HT release were normal.

The patient's platelets demonstrated normal levels of glycoprotein (GP)IIb/IIIa and GPIb/IX expression by flow cytometry. The direct platelet immunofluorescence test was positive. Her serum contained anti-GPIIb/IIIa. The platelet eluate contained auto anti-GPIIb/IIIa, GPIb/IX and GPIa/IIa [GTI PAK 12 enzyme-linked immunosorbent assay (ELISA); Quest, UK]. In mixing experiments, control platelets failed to aggregate to the agonists mentioned above when exposed to the patient's plasma. Flow cytometry studies on activated platelets incubated with normal and patient's plasma were conducted using fluorescein isothiocyanate-conjugated anti-PAC I (activated GPIIb/IIIa), phycoerythrin (PE)-conjugated anti-P selectin and PE-conjugated anti-CD4 1 (non-activated GPIIb/IIIa) (Becton Dickinson, UK). Analysis of gated platelets showed that the patient's antibody bound to both activated and non-activated GPIIb/IIIa.

A diagnosis of acquired Glanzmann's thrombasthenia was made. A month after her initial presentation, she was admitted with severe gastrointestinal bleeding. No focal point was obvious despite extensive investigation. Prednisolone was commenced at 60 mg/d and subsequently azathioprine 100 mg/d was added. Despite this, gastrointestinal blood loss continued. Mixing experiments suggested probable improvement in platelet function with dilution of her plasma (Fig 1) and, therefore, plasma exchanges were commenced. Twelve plasma exchanges (latterly through a femoral catheter kept patent with intraluminal heparin) resulted in normalization of her platelet function and bleeding time with cessation of gastrointestinal bleeding.

A week after insertion of the femoral line, her platelet count fell to 50×10^9/l and she developed a large femoral deep venous thrombosis, extending into the right iliac vein. An ELISA demonstrated a heparin-platelet factor 4 antibody with complete blockade by excess heparin and a diagnosis of heparin-induced thrombocytopenia with thrombosis was made. Anticoagulation was commenced with hirudin after removal of the catheter and a mechanical right iliac vein thrombectomy was performed successfully. Hirudin was continued keeping an APTT ratio of 2 for 2 weeks. Warfarin therapy was not initiated.

Her gastrointestinal bleeding has not recurred. She is now well 18 months after her presentation, and her immunosuppressive treatment was tapered and stopped over 1 year. Her recent platelet function was normal and the platelet immunofluorescence tests were negative.

Review of literature has revealed eight cases of acquired Glanzmann's thrombasthenia, most of them being associated with lymphoproliferative disorders (reviewed by Malik *et al*, 1998). The successful outcome in this case was probably related to instituting plasma exchanges while awaiting the effects of immunosuppressant therapy. Recombinant VIIa has also been used to treat bleeding in Glanzmann's thrombasthenia (d'Orion *et al*, 2000; Patel *et al*, 2001).

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Keywords: acquired Glanzmann's thrombasthenia, plasma exchange, heparin-induced thrombocytopenia, mechanical thrombectomy.

MANAGEMENT DILEMMA OF CARDIOPULMONARY BYPASS IN PATIENTS WITH TYPE II HEPARIN-INDUCED THROMBOCYTOPENIA

We would like to share our most recent experience in carrying out cardiopulmonary bypass (CPB) in a patient with type II heparin-induced thrombocytopenia (HIT II).

A 77-year-old woman was referred to our cardiothoracic unit with right axillary vein thrombosis secondary to right atrial myxoma, an unusual presentation of a rare tumour. The patient was started on low molecular subcutaneous heparin (tinzeparin; innohep; Leo Laboratory, UK) at 175 IU/kg once daily, which caused the platelet count to fall from 261 to 51×10^9/l over 10 d. The subsequent enhanced enzyme-linked immunosorbent assay (GTI, Brookfield, USA), for detection of antibodies directed against complexes of platelet factor-4 and polyvinyl sulphonate, was positive. Heparin was stopped and the operation was postponed. Fortunately, in this case, there was no associated thrombosis as part of the HIT type II syndrome.

For the management of CPB, this case posed some clinical dilemmas. We considered three different strategies in the management of this patient. The first was to proceed with the surgical resection under conventional CPB with concomitant administration of a potent anti-platelet aggregation agent such as iloprost (Addonizio *et al*, 1987), but this was deemed a dangerous option as iloprost can disturb the haemodynamics owing to its vasodilatory action. The second option was to allow the HIT antibodies to reach an undetectable level, which usually takes about 100 d, before undergoing CPB with heparin re-exposure (Warkentin & Kelton, 1998). This option entailed a considerable delay with a significant risk of recurrent thromboembolic complications during the long seroconversion period. The third strategy was the avoidance of a secondary immune response by performing CPB utilizing a heparin substitute such as recombinant hirudin (r-hirudin) as the anti-coagulant agent for the CPB (Riess *et al*, 1996).

On the balance of risks and benefits of each strategy, the third option seemed the most clinically attractive but there is currently no available agent to reverse the anti-coagulant effect of r-hirudin at the end of CPB, raising the risk of intra- and postoperative haemorrhage.

The operation was carried after a 12 d delay. The preoperative platelet count was 224×10^9/l. A heparin-free CPB circuit was used; non-heparin-coated vascular catheters were used in this patient. All catheter rinsing fluids were heparin-free. r-Hirudin (lepirudin; refludan; Hoechst, France) was used as the anti-coagulant for the CPB with the Ecarin Clotting Time (ECT) measured every 15 min and the corresponding concentration of r-hirudin calculated; 0·25 mg/kg of body weight was used to prime the CPB circuit and 0·2 mg/kg body weight was given followed by 0·5 mg/min as a continuous infusion. The r-hirudin concentration was maintained in the range of 3·5–4·5 μg/ml. The resection was carried out successfully. Because of its short half-life (approximately 1 h), r-hirudin infusion was stopped at the end of the procedure, allowing the plasma level to decline rapidly and its anticoagulant effect to be reversed before chest closure. The intraoperative blood loss was 290 ml. There was no abnormal bleeding or fibrin formation in the extracorpeal circuit during the CPB. The postoperative course was unremarkable with no bleeding or thrombotic complications. The patient was extubated and chest drains were removed at 6 and 23 postoperative hours, respectively, with a total blood loss of 320 ml. The patient was discharged to the referring hospital on postoperative d 6 for cardiac rehabilitation.

Type II heparin-induced thrombocytopenia poses several problems for the conduct of CPB but, from our limited experience and the available data, r-hirudin could play a role in the management of CPB if strict control of the ECT is exercised. More research and clinical trials will hopefully lead to better insight and clinical practice.

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Keywords: heparin, heparin-induced thrombocytopenia, cardiopulmonary bypass, hirudin, cardiac surgery.