

LESSONS FROM A SYMPOSIUM ON HAEMATOLOGY HELD IN THE COLLEGE ON 5 MARCH 1998*

Lynn M. Manson,[†] *Department of Haematology, Royal Infirmary of Edinburgh*

There are few medical practitioners who can avoid exposure to haematological problems in their daily practice, irrespective of their line of specialisation, and this was reflected in the diversity of medical practitioners who attended the Symposium, *Haematology - Rational Answers to Recurring Questions* held in the College in early March. It was therefore appropriate that the speakers at the Symposium attempted to provide practical, evidence based solutions to both common and complex rarer haematological problems which are faced recurrently by many clinicians.

WHO WOULD BENEFIT FROM A THROMBOPHILIA SCREEN?

A single point mutation in the factor V gene (factor V Leiden) is the cause of approximately 95% of cases of activated protein C resistance (APCR)^{1,2} and is currently the commonest inherited thrombophilia; it is found in 5-10% of asymptomatic individuals in North Europe and the USA but is less common in Asia and Africa. It is associated with an increased risk of venous thromboembolism (VTE): homozygotes have a 90-fold increased risk of VTE, and heterozygotes an 8-fold increased risk, when compared with non-affected individuals. In the presence of another prothrombotic risk, such as the combined oral contraceptive pill (COCP), the risk induced by the two conditions is cumulative. Females taking the COCP have up to a 4-fold greater risk of suffering a VTE compared with female non-users, but female COCP users who are factor V Leiden heterozygous have a 35-fold increased risk of experiencing this complication.³ This suggests that there may be scope for screening, but if so, the question is, 'Who should be screened?'

The success of a screening programme is dependent upon whether knowledge of the carrier status is advantageous to the asymptomatic individual. Although those with the factor V Leiden abnormality are at increased relative risk of VTE, the absolute risk of such an event occurring, the probability that this will be life-threatening, the predictability of concurrent risk factors, and the risk of haemorrhage from long-term anticoagulation, must all be ascertained to determine if screening can be proposed as a means to reduce morbidity and mortality.

The risk of VTE in factor V Leiden heterozygotes depends upon the manner in which carriers present, and the presence of concomitant prothrombotic risk factors (such as immobilisation, major surgery, the COCP and pregnancy). Asymptomatic individuals incidentally found to be factor V Leiden heterozygous appear to be at less risk of future VTE than age-matched symptomatic individuals who are factor V Leiden heterozygous. Twice as many women who are factor V Leiden heterozygous and have had a VTE outwith pregnancy will experience a further VTE in the course of a

*A list of speakers and the titles of their papers presented at this symposium is recorded in *Proceedings* Vol.28, p.300.

[†]Senior Registrar in Haematology.

pregnancy or the puerperium compared with their asymptomatic first-degree relatives who are found to be factor V Leiden heterozygous and become pregnant.⁴ In general, asymptomatic carriers have four times the risk of VTE compared with asymptomatic non-carriers, but in absolute terms the risk is small for both groups.

However, the risk in asymptomatic carriers increases with age, until there is a significantly greater absolute risk in asymptomatic carriers compared with non-carriers over 50 years of age.

Individuals who are factor V Leiden heterozygous are also at increased relative risk of suffering a pulmonary embolus compared with normal individuals, but they are only 3.3 times more likely to experience this problem compared with unaffected individuals as the increase in risk is only 50% that seen with deep vein thrombosis.

The incidence of anticoagulant-induced major haemorrhage is 1-3% with a 0.2-0.6% incidence of fatal haemorrhage, but the risk of haemorrhage increases with age and the rate of major haemorrhage increases by 57% with every 10-year increase in age. The optimal duration of therapy in individuals requiring long-term anticoagulation remains unclear. Overall, the risk of treatment-related haemorrhage is greater than the benefit of PE prevention, except in the 15-40 year age group when anticoagulation is beneficial, thus only a small subgroup of young individuals with VTE will benefit from long-term anticoagulation. Even although the relative risk of PE in factor V heterozygotes is twice that in non-carriers, the risk of major haemorrhage with long-term anticoagulation remains greater than the benefit of PE prophylaxis, so it is therefore dubious whether factor V Leiden screening would be beneficial in PE prevention.

Routine screening of women for factor V Leiden prior to commencing the COCP is also of little benefit. To prevent one woman who is factor V Leiden heterozygous suffering a fatal pulmonary embolus, 400,000 women would need to be screened at a (conservative) cost of £8,000,000, which does not include the non-financial expense of pre-test counselling or the psychological stress experienced by the individual undergoing screening. Routine screening would also deprive thousands of women of effective oral contraception, and expose them to the risks of unwanted pregnancies and pregnancy-associated VTE.

What advice should be offered to an individual with a positive family history of VTE who wants to be prescribed the COCP but has been found to be factor V Leiden heterozygous on screening? If the patient has a concomitant thrombophilic condition, she should be advised to avoid oestrogen-containing preparations. The correct advice for a woman who is only factor V Leiden heterozygous is more contentious. Each case should be treated individually, and the relative and absolute risks of the COCP in a factor V Leiden heterozygous individual must be discussed, as well as the risks of alternative forms of contraception. The final decision can then be left up to the individual, who can request further guidance from the clinician as needed.

It will only become clearer which patient subgroups will benefit from screening programmes as experience with the newer inherited thrombophilias grows. Current evidence suggests that mass screening programmes are not beneficial and screening should be reserved for individuals with recurrent VTE or a strong family history of VTE, and that this testing should be supported by appropriate pre-test patient counselling.

Bullet points:

- Thrombophilia screening is not recommended for asymptomatic individuals.
- Screening should be reserved for those with recurrent VTE or a positive family history.
- Always consider each patient individually.
- Take a full history to determine if prothrombotic risk factors are present.
- Long-term anticoagulation is not without risk.
- The incidence of anticoagulant-induced haemorrhage increases significantly with age.
- Optimal duration of therapy has yet to be determined.
- Routine screening for the factor V Leiden polymorphism prior to commencing the combined oral contraceptive pill is of little benefit.

ANTIPHOSPHOLIPID ANTIBODIES (APA): RELEVANCE TO CLINICAL PRACTICE

The clinical manifestations of primary and secondary antiphospholipid syndrome (APS) are similar, and include arterial and venous thrombosis, small vessel and placental vessel occlusion. Antiphospholipid antibodies (APA) occur in many disorders (autoimmune conditions, infections [viral, *Pneumocystis carinii*, syphilis], lymphoproliferative disorders), and after exposure to certain drugs (40% of individuals on chlorpromazine develop APA after one year). Laboratory results often confirm the clinical suspicion of the presence of APA, but APS can be difficult to diagnose because of its ambiguous presenting symptoms and unhelpful laboratory tests, such as when a lupus anticoagulant is absent and only low-titre anticardiolipin antibodies are present. In addition, APA are found in 1% of healthy individuals and are of little clinical significance, and so there is no benefit in screening for APA in the absence of a suggestive history.

APA occur in children, and most likely cause a significant proportion of unexplained cases of stroke and pulmonary embolism in this age group. They invariably result in a severe prothrombotic tendency following varicella infection, in contrast to their incidental presence in adults after infections (such as syphilis).

Two laboratory assays are used to detect the presence of a lupus anticoagulant, as some anticoagulants may be detectable using the DRVVT (dilute Russell's viper venom test) but not the KCCT (kaolin-cephalin clotting time), and vice versa. Current tests used to detect ACA are not ideal, as low-titre antibodies with a significant *in vivo* effect may not be detected *in vitro* and conversely, the clinical relevance of *in vitro* low-titre ACA is unclear. A new ELISA-based anti- β_2 glycoprotein 1 (anti- β_2 GP1) assay may improve the diagnostic power of current tests. Preliminary results suggest that individuals with weakly positive DRVVT/KCCT results or low-titre ACA but no abnormality using the anti- β_2 GP1 assay are at low risk of thromboses.

The pathogenic role of APA in thrombus formation is unclear; they are found in asymptomatic individuals and can occur with other autoantibodies. It remains to be determined if they are causal or simply surrogate markers of the prothrombotic state.

Approximately 5% of patients with APS suffer recurrent thromboembolic events but only 0.95% of individuals with coincidental APA have a thrombotic episode, which is comparable to the background population rate.⁵ This suggests a beneficial role for long-term anticoagulation in the treatment of APS, but potential benefits must be weighed against the 0.5% incidence of anticoagulation-related fatal haemorrhage. In those who merit long-term anticoagulation, a target INR of >3 is required to prevent recurrent thromboembolism, but the reduced thromboprophylaxis associated with a target INR of < 3 may be an acceptable compromise in individuals

in whom the risk of cerebral haemorrhage is greater.

To optimise treatment, each case must be assessed individually and therapy adapted according to the individual characteristics. Therapeutic strategies to consider include long-term anticoagulation for individuals with a lupus anticoagulant >40 U/L who suffer a life-threatening thromboembolism, long-term warfarin (escalate INR to target of 3.5) for recurrent thromboses, and warfarin for three months (target INR 2.5) after the first (non-life-threatening) thrombosis.

Bullet points:

- No benefit is derived in screening for APA in the absence of a history suggestive of antiphospholipid syndrome.
- Currently-available laboratory tests to detect APA are not ideal.
- Diagnostic power will be improved by new ELISA-based assay.
- The pathogenetic role of APA in thrombus formation remains unclear.
- Potential benefits of long-term anticoagulation in APS must be weighed against the risk of anticoagulant-associated haemorrhage.
- Optimise therapy, assess each case individually and tailor treatment accordingly.

THROMBOPHILIA, VENOUS THROMBOEMBOLISM (VTE) AND PREGNANCY

When data from objectively-confirmed VTE are analysed, the incidence of non-fatal pregnancy-associated VTE is 0.86/1,000 deliveries. The incidence of deep vein thrombosis (DVT) is 0.71/1,000 deliveries, of which 0.50/1,000 are antenatal and 0.21/1,000 are puerperal events; relatively few pulmonary emboli occur (0.15/1,000 pregnancies), and similar numbers occur ante- and post-partum.⁶

The puerperium remains the period of greatest thrombogenic potential because in terms of women-years at risk, the incidence of puerperal thrombosis is three times greater for DVT (antenatal 0.065/100 women years v puerperal 0.18/100 women years) and almost eight times greater for PE (antenatal 0.009/100 women years v 0.07/100 women years). More episodes of pregnancy-associated VTE will be prevented per week of anticoagulant therapy in the puerperium than in the antenatal period.

Pregnancy *per se* is a prothrombotic risk factor but acquired risk factors during pregnancy further increase that risk (increasing age [>35 years] and parity [para 4+], weight [> 80 kg at booking], immobility, coexistent medical problems, APS). Caesarean section, complicated vaginal delivery and previous VTE also are risk factors. Inherited thrombophilic defects (antithrombin III [AT III], protein C and S deficiencies, factor V Leiden and PT 20210A mutations) all increase the risk of pregnancy-associated VTE but by differing degrees; from 1 in 2.8 with type I AT III deficiency to 1 in 113 with protein C deficiency. The thrombotic risk for an asymptomatic woman who is factor V Leiden heterozygous is low (1 in 400-500 factor V Leiden-positive pregnancies) and usually additional clinical risk factors are present when VTE occurs in these individuals, so random screening for the mutation in early pregnancy is not beneficial.⁶

A significant number of pregnancy-associated VTE will be difficult to predict and prevent as 30-40% of events have no detectable clinical risk factors. However, a family history of VTE in a first-degree relative is more common in women with pregnancy-associated VTE compared with controls, and so a full family history is essential in antenatal thrombotic risk screening in addition to identification of acquired thrombotic risk factors.

Female children from thrombophilic families require special consideration. These girls should be screened at 12 years of age: affected girls should be offered pregnancy and contraception counselling, and facilities for early pregnancy confirmation (by 5-6 weeks' gestation) should be available.

Pregnancy-related thromboprophylaxis strategies are currently Grade C recommendations based on level IV evidence.⁷ Pregnant women should undergo thrombotic risk assessment, and thromboprophylaxis should be initiated according to risk stratification with locally-agreed strategies available for the delivery period, but it is crucial that each pregnancy is considered individually and the woman looked after at a specialist centre. Low molecular weight heparin (LMWH) prophylaxis from early pregnancy until 12 weeks post-delivery is recommended for those at very high risk (AT III deficient or on long-term anticoagulation), with appropriate anticoagulant and platelet monitoring, and the option of warfarin use post-partum. Less intensive LMWH prophylaxis from mid-trimester until six weeks post-partum is recommended for high-risk individuals (previous history of VTE, protein C deficiency, factor V Leiden or PT 20210A homozygous, more than one prothrombotic risk factor). Clinical and radiological monitoring is recommended for medium-risk pregnancies (no personal but strong family history of VTE, protein S deficiency, factor V Leiden or PT 20210A heterozygous).

Bullet points:

- The puerperium is the period of greatest thrombogenic potential.
- More episodes of pregnancy-associated VTE will be prevented with anticoagulant therapy in the puerperium than in the antenatal period.
- Each woman should undergo a full thrombotic risk assessment and acquired thrombotic risk factors noted.
- Random screening for the factor V Leiden polymorphism in early pregnancy is not beneficial.
- Up to 40% of VTE events have no identifiable risk factors.
- A significant number of pregnancy-associated VTE will be difficult to predict and prevent.
- Thromboprophylaxis during pregnancy should be monitored at a specialist centre.
- Female children from thrombophilic families require special consideration.

INVESTIGATING AND MANAGING THE PATIENT WITH A HIGH PLATELET COUNT

A high platelet count may be an inherited or acquired disorder. A heterogeneous group of genetic mutations is implicated in the former disorders and include abnormalities of the thrombopoietin (Tpo) gene and mutations in the Tpo receptor. Acquired disorders may be primary (myeloid malignancies: chronic myeloid leukaemia, polycythaemia rubra vera [PRV], myelofibrosis, essential thrombocythaemia [ET], myelodysplasia) or secondary (reactive thrombocytosis due to inflammation, neoplasia, trauma, bleeding).

The use of bone marrow trephine appearances, clonality, Tpo quantification and megakaryocyte colony status as positive diagnostic tools in ET has been assessed. A hypercellular marrow trephine with clusters of large, pleomorphic megakaryocytes is characteristic of ET, and not found in reactive thrombocytoses (smaller, non-clustering

megakaryocytes). As similar appearances are found in PRV, marrow trephine appearances will only help in distinguishing reactive thrombocytoses from myeloproliferative disorders.

Polymorphisms using X-linked probes have been studied in women, and clonal granulocyte, platelet/polyclonal T lymphocyte patterns in peripheral blood analysis are found in 60-70% of females with ET, but as 25% of haematologically normal elderly females show a similar pattern, this is of little diagnostic worth.

Unexpectedly elevated Tpo levels have been reported in ET, possibly as a result of Tpo receptor abnormalities (as shown by reduced Tpo receptor levels); confirmation of this finding and levels in reactive thrombocytoses are needed before the positive diagnostic power of this finding can be evaluated. Both spontaneous EFU-burst and megakaryocyte colonies are found in ET but not in reactive thrombocytoses, and although these methods have diagnostic value they are not yet routinely available. Thus, the diagnosis of ET continues to be predominantly one of exclusion.

When ET is diagnosed, therapy is essential as 4% of low-risk and 12% of high-risk individuals suffer a thrombotic event each year (risk factors include increasing age, platelet count >1,000.109/L, previous thrombotic event). Intervention is beneficial as cytoreductive therapy produces a six-fold reduction in vascular occlusion in high-risk patients, but drug-induced side-effects must be considered as some studies have shown that cytoreductive therapy doubles the incidence of acute leukaemic transformation in selected patients, and its effect on fibrotic development is unknown.

The MRC PT1 trial aims to determine optimal management for ET. Patients will be placed into one of three groups, having a 'low', 'intermediate' or 'high' risk of vascular occlusion. Low-risk patients will receive aspirin alone, intermediate-risk will be randomised to receive either aspirin or aspirin and hydroxyurea, and high-risk patients will be randomised to either aspirin and hydroxyurea or to aspirin and anagrelide. The study's primary objective is to examine the outcome in terms of vascular occlusion and the secondary objective is to examine the incidence of haematological transitions towards malignancy; the effect of the treatment modalities on quality of life will also be determined. Approximately 500-600 patients will be required in each of the randomised components of the study. Recruitment will take three to five years and a median follow-up of three years is likely to be necessary to answer the primary objectives.

Bullet points:

- The diagnosis of ET (essential thrombocythaemia) is primarily one of exclusion.
- Cytoreductive therapy in ET is essential to reduce the thrombotic risk.
- In some patients such therapy may increase acute leukaemic transformation and fibrotic development.
- The MRC PT1 Trial will determine the optimal management of ET.

TREATMENT OF VENOUS THROMBOEMBOLIC DISEASE (VTED): IMPLICATIONS FOR THE NEXT CENTURY

Deep venous thrombosis (DVT) and pulmonary embolism (PE) can be regarded as different ends of the continuous spectrum of venous thromboembolic disease (VTED). This condition is associated with significant morbidity, with a cumulative recurrence

rate of 25% in the eight years after a first episode of VTED. Although VTED-associated mortality *per se* is low, malignancy is an important cause of mortality in individuals suffering apparently idiopathic VTED as the malignancy is often occult at presentation of the VTED, and only becomes apparent at short-term follow-up. Despite this strong association, no current screening programmes facilitate detection of occult malignancies and improve life-expectancy, and it is crucial that malignancies are identified whose early detection would result in health, economic and life-expectancy benefits.

VTED-associated morbidity and mortality can be minimised if initial therapy is started promptly and therapeutic anticoagulation achieved within 24-48 hours of therapy introduction. Until recently, unfractionated heparin (UFH) was used widely for the prophylaxis and treatment of VTED. It is often difficult to achieve therapeutic anticoagulation with UFH because of marked variation in dose-response necessitating frequent laboratory monitoring of the anticoagulant response. Its clinical usefulness is further limited by its short half-life, its poor bioavailability at low doses, and the need for parenteral administration, usually by continuous intravenous infusion within hospital. The low molecular weight heparins (LMWHs) overcome these difficulties as they give a more predictable anticoagulant response to weight-adjusted doses and so do not require laboratory monitoring; their longer half-lives and greater bioavailability at low doses compared with UFH mean that they can be administered once or twice daily by subcutaneous injection. They are as safe and effective as UFH in the prophylaxis and treatment of VTED in hospital and at home, and in the treatment of unstable angina, and will probably soon replace UFH as the anticoagulant of choice for many clinical conditions.

The safety and efficacy of the LMWHs have been confirmed by a Cochrane Collaboration-initiated meta-analysis of 13 randomised studies over a ten-year period that compared the use of UFH with LMWH for the treatment of objectively-proven VTED. Pooled analysis of all the trials showed a non-significant reduction in recurrent VTED in those treated with LMWH compared with UFH, a significant reduction in the occurrence of major haemorrhage in the first ten days of therapy in those treated with LMWH, and a significant reduction in mortality in individuals who received LMWH, this benefit was limited to individuals with coexistent malignancy, as there was no difference in mortality between groups treated with LMWH or UFH who did not have a malignancy. It was stressed that each patient must be adequately assessed on an individual basis, the diagnosis must be objectively confirmed, and facilities must be available for the initiation and maintenance of therapy in order to obtain the benefits of out-patient LMWH therapy.

The optimal duration of oral anticoagulation remains undetermined. Significantly more recurrent thromboembolic events are observed in individuals treated for six weeks compared with those treated for six months. The recurrence rate is greater in individuals treated for six months after a second thromboembolic event compared with those who receive anticoagulation indefinitely, but there are almost three times fewer major haemorrhagic events in the fixed-period group.

In an attempt to minimise both recurrent thromboembolic and haemorrhagic events, the following guidelines have been proposed, but therapy should be tailored to meet each patient's individual requirements:

- i) DVT/PE and reversible risk factor: 4-12 weeks' therapy;
- calf DVT: 4-6 weeks' therapy;
- proximal DVT/PE: 12 weeks' therapy;

- | | | |
|------|--|--|
| ii) | DVT/PE and no reversible risk factor: | 6 months' therapy; |
| iii) | DVT/PE and activated protein C resistance: | 6 months' therapy; |
| iv) | DVT/PE and other inherited thrombophilia: | 1-2 years' therapy (consider indefinite anticoagulation if coexistent prothrombotic risk factors). |

New anticoagulants continue to be developed in an attempt to discover an oral preparation with optimal inhibition of clot-bound thrombin which will be safe and effective in the home treatment of VTED. A synthetically-produced antithrombin III-pentasaccharide binding sequence is currently being evaluated in prophylaxis and therapeutic studies and may be available clinically in three to four years. It is much cheaper to produce than the LMWHs and although it is given subcutaneously, it can be absorbed orally.

Bullet points:

- Occult malignancy is an important cause of apparently idiopathic VTED.
- The malignancy only becomes apparent at short-term follow-up.
- Therapeutic anticoagulation must be achieved within 24-48 hours of initiation of therapy to minimise VTED-associated morbidity and mortality.
- LMWHs as compared to UFH are more beneficial because of a longer half-life, more predictable dose-response, subcutaneous administration and absence of a need for regular laboratory monitoring.
- Optimal duration of oral anticoagulation remains undetermined.
- Therapy must be tailored to meet each patient's needs.

CLINICAL USE OF GROWTH FACTORS

The current guidelines which recommend specific indications for the use of haemopoietic colony-stimulating factors (CSF) or growth factors are based on best clinical practice.⁸ However, the economic implications of widespread CSF use must be considered along with their clinical benefits, and in practical terms, guidelines based on clinical and economic considerations are likely to be most widely accepted.⁹

Use of haemopoietic growth factors in conventional lymphoma therapy

Routine CSF support to prevent neutropenic sepsis is recommended only for those patients with a >40% risk of developing sepsis. Risk factors for this complication include advanced age, retroviral disease, extensive marrow involvement, use of intensified regimens, poor performance status, and sepsis in a preceding course. Use of CSF support in the treatment of neutropenic sepsis and for maintenance and enhancement of dose intensity remains empirical as randomised trials to determine its benefit in these situations have not yet been undertaken.

Use of CSF after myeloid malignancy induction therapy

It is recommended that CSF are used to shorten the duration of neutropenia after the completion of remission induction therapy in older patients (>55 years). The evidence for this recommendation is conflicting but well-substantiated data show that post-chemotherapy CSF in older AML patients results in a small acceleration in neutrophil recovery which may provide some clinical benefit, and CSF therapy should be delayed until post-chemotherapy aplasia has been documented at day +10 post-therapy, providing financial savings. A similar effect may be seen in those <55 years, but in younger AML patients with a lower treatment-related mortality the role of CSF remains unproven and their use cannot be recommended outwith a clinical trial.

Use of CSF for priming of leukaemic cells

Despite good *in vitro* evidence to the contrary, to date none of the clinical studies using CSF for priming has resulted in any improvement in CR or survival rates; the use of CSF given before and/or with chemotherapy for priming effects should not be undertaken outwith a clinical trial. This disparity may have occurred because CSF can also act as viability factors for leukaemic cells and can suppress apoptosis in the presence of cytotoxic drugs such as daunorubicin, possibly by inducing proteins such as bcl-2 which act to suppress apoptosis induced by anti-cancer drugs.

Use of CSF in autologous transplantation

CSF can be used to reduce the duration of neutropenia, shorten the number and severity of infectious complications and so decrease the period of hospitalisation and reduce cost. There is anecdotal evidence that G-CSF may be more effective and better tolerated than GM-CSF, but neither appears to adversely affect procedure-related toxicity or relapse rate in the autologous setting. The use of CSF post-autograft in AML is not recommended as no studies as yet have evaluated the theoretical risk of stimulating residual leukaemia in this setting. The optimal dose, timing and duration of administration of CSF are not established. Delaying the onset of CSF support until day +6 post-transplant and reducing the dose to 50 mg/m² have both been shown to reduce post-transplant in-patient stay with significant economic savings.

Use of CSF in allogeneic transplantation

CSF enhance neutrophil recovery and function when given after allogeneic sibling marrow and peripheral stem cell transplantation in adults (which reduces cost because of earlier hospital discharge) and G-CSF appears to have a similar effect in children. The optimal cost-effective dose and schedule for CSF administration is not yet established. Randomised trials are needed to confirm the impression that CSF appear to have a beneficial role following unrelated donor transplantation. Concerns about the use of CSF in the allogeneic setting and the theoretical risk of increased GVHD appear ill-founded, although there may be an increased risk of chronic GVHD in older patients.

Use of CSF following delayed/poor engraftment

A trial of GM-CSF is warranted with delayed or inadequate neutrophil engraftment (ANC <0.2.10⁹/L by D+28) after either autologous or allogeneic transplantation. In patients who have already received G-CSF, a switch to GM-CSF is indicated as G-CSF is less effective than GM-CSF in delayed/inadequate engraftment.

Use of CSF for mobilisation of peripheral blood stem cells (PBSC)

Evidence suggests that PBSC mobilised by CSF and chemotherapy are superior to those mobilised by CSF alone (in terms of CD34⁺ cell yield and subsequent speed of engraftment) but combination treatment may be inappropriate in some circumstances (paediatric practice, normal donors). Potential predictive factors for inadequate PBSC mobilisation using chemotherapy and CSF include the amount of previous chemotherapy, previous radiotherapy and underlying Hodgkin's disease or myeloma. The use of other recombinant CSFs (IL-3, stem cell factor) is being evaluated but these will only be of benefit if they further increase the yield of CD34⁺ cells harvested, further accelerate engraftment or permit mobilisation in patients who have failed or are likely to fail using standard regimens.

Mobilisation of normal donors using CSF

G-CSF has an excellent safety record and minimal toxicity when compared with GM-CSF, and can be used safely to mobilise sufficient stem cells into the peripheral blood of normal donors for subsequent harvest and allogeneic sibling transplantation. The use of PBSC for unrelated donor transplantation has been reported but is not yet widely available.

Bullet points:

- Routine CSF (colony-stimulating factors) prophylaxis against neutropenic sepsis in conventional lymphoma treatment is recommended only for patients with greater than 40% risk of developing sepsis.
- Post-chemotherapy CSF will accelerate neutrophil recovery and improve clinical outcome in older (> 55 years) AML patients only.
- The *in vivo* use of CSF for leukaemic cell priming has, to date, not improved survival, despite encouraging *in vitro* results.
- A trial of GM-CSF is warranted with delayed or inadequate neutrophil engraftment after either autologous or allogeneic transplantation.
- Predictive factors for inadequate peripheral blood stem cell mobilisation include amount of previous chemotherapy, previous radiotherapy, and underlying Hodgkin's disease or myeloma.

CURRENT MANAGEMENT OF CHRONIC LYMPHATIC LEUKAEMIA (CLL)

The disease stage, WBC and age of patient at presentation, the response to therapy and the lymphocyte doubling time are important prognostic factors in determining optimal management of chronic lymphatic leukaemia (CLL). In general, good responders do better than poor responders, but it is worthwhile persevering with therapy in initial poor responders as some individuals who initially fail to respond will eventually obtain a sustained response to a particular treatment.

The most important prognostic factor is the disease stage, now generally described using the Binet classification¹⁰ (Table 1). Fifty percent of individuals who are Binet stage A at presentation will die of causes other than CLL, but the majority of those with stage B and C disease will die from their disease. Results from the MRC CLL3A trial have confirmed that early treatment of stage A patients may be deleterious to their outcome. The WBC doubling time should be assessed,

TABLE 1
International Working Party classification of chronic lymphocytic leukaemia.¹⁰

	Organ enlargement [†]	Haemoglobin* (g/dL)	Platelets* (x 10 ⁹ /L)
Stage A	< 3	≥ 10	≥ 100
B	≥ 3	≥ 10	≥ 100
C	not considered	< 10 and/or	< 100

*Secondary causes of anaemia (e.g. iron deficiency) or autoimmune haemolytic anaemia, or of thrombocytopenia must be treated before staging.

[†]One area = lymph nodes >1cm in neck, axillae, groins, or spleen or liver enlargement. Bilateral involvement at a site is counted as one site.

and treatment reserved for those with a rapid doubling time (<1 year) as this subgroup progresses faster than those with a doubling time of >1 year. Stage A patients should be categorised into the appropriate French sub-stage of A^I or A^{II} (stage A^{II}: Hb<12 g/dL or WBC>30.10⁹/L). Both rapid WBC doubling time and French sub-stage A^{II} are independent prognostic features, with the WBC doubling time having greater prognostic significance. Data from the MRC CLL 1, 2 and 3 trials have shown a sex advantage to survival; not only do fewer females develop CLL, but those with the disease have a less aggressive form and respond better to equivalent therapy than men.

No combination regimen has been shown to be superior to chlorambucil (which remains the mainstay of therapy) including chlorambucil and prednisolone (MRC CLL 2), COP (cyclophosphamide, vincristine, prednisolone: MRC CLL 1), CHOP (COP + doxorubicin: French and other trials), and chlorambucil and epirubicin (MRC CLL 3). What may be important in determining response to therapy and survival is the dose of chlorambucil administered, and this has varied amongst the different trials, from a single dose of 40 mg/m² in the CALGB trial (3% CR rate) to 10 mg/m² daily for six days in the MRC CLL3 trial (a five-fold increase in CR rate was achieved with only a 50% dose increase, with 15% CR rate and 56% PR rate). Up to three times the total dose used in MRC CLL3 (150-180 mg/m²) has also been assessed and response rates higher than CHOP and comparable with fludarabine (59% CR, 28% PR) have occurred but this is accompanied by toxicity and unproven impact on survival.

The nucleoside analogues (such as fludarabine [FDR]) have given higher response rates than chlorambucil and combination therapy in the treatment of specific cases of CLL and low-grade lymphoma. Single agents have less toxicity than combination therapy and appear to facilitate autologous transplantation in younger patients. FDR offers potential for synergy in combination with cyclophosphamide, mitozantrone and epirubicin but offers no additional benefit when combined with chlorambucil, and the combination of FDR/cyclo appears to be better than FDR alone or FDR/mito. The MRC CLL 3NR trial has established that FDR is as successful as chlorambucil in the treatment of patients with primary resistance to chlorambucil and combination therapy (16% CR, 56% PR, 27% NR) although no benefit in overall survival has been seen yet.

MRC CLL4 will attempt to determine the optimal dose of chlorambucil and establish new therapeutic strategies by comparing chlorambucil (increased dose of 10 mg/m² for seven days) with FDR, either alone (25 mg/m² for five days) or in combination with cyclophosphamide (FDR 25 mg/m² for three days + cyclophosphamide 250 mg/m² for three days), analysing survival, response rates, disease-free survival, toxicity and quality of life.

Other therapeutic options exist for CLL but many are in the preliminary stages of clinical use and initial benefits may not be sustained. Comparative trials are needed to determine the clinical applicability of cladribine and CAMPATH (monoclonal antibody with cytotoxic anti-lymphocyte activity used for *in vivo* and *ex vivo* lymphocyte reduction). The role of high-dose therapy and autologous transplantation in young individuals with progressive CLL is currently under MRC study and initial results show a 70% response rate; responses in refractory patients have been poor but results in early disease are more promising. There is no value in *in vitro* purging and it is accepted that some harvested diseased cells may be given back to the recipient but appear not to be detrimental to outcome.

Bullet points:

- Prognostic factors in determining optimal management of CLL include disease stage, WBC, age of patient at presentation, response to therapy, and the lymphocyte doubling time.
- No combination regimen has been shown to be superior to chlorambucil.
- Response to therapy and survival may depend upon the dose of chlorambucil administered.
- Some who initially fail to respond to therapy will eventually achieve a sustained response, so persevering with therapy is important.
- Nucleoside analogues are indicated for specific cases of CLL and low-grade lymphoma.
- These analogues may offer survival advantage when used in combination with other agents.
- High-dose therapy and autologous transplantation should be offered early to young patients with progressive disease.

MOVING GENE THERAPY FROM LABORATORY TO PATIENT¹¹

The haemophilias have become a major target for gene therapy but it is unlikely that present technology will provide a total cure for haemophilia through a life-long gene transfer that will ensure continuing production of coagulation factor to normalise levels. An acceptable and feasible goal is to achieve a plasma level of 5-10 U/dl of the relevant coagulation factor by using a simply-administered 'gene treatment' every few months; this would protect against spontaneous bleeding and provide a substantial improvement in lifestyle.

Methods of gene transfection fall into two main categories: gene delivery using recombinant viral vectors, and physical gene delivery. Cells can be modified by vector systems either outside the body (*ex vivo* modification) or within the body (*in vivo* modification). Potential viral vectors include retroviruses, adenoviruses, parvo/adeno-associated viruses and herpes viruses. Most viruses used for gene transfer have had

genes that confer virulence removed, and coagulation factor VIII or IX genes are substituted for viral genes. Physical gene delivery systems are attractive alternatives to recombinant viral vectors. Methods under evaluation include direct gene transfer by injection, liposome encapsulation and receptor.

In haemophilia, most of the work has been performed using factor IX cDNA because the large size of the factor VIII gene has placed constraints upon its transfer by available vector systems, and factor VIII is less stable than factor IX. Studies on factor VIII have used smaller B domain-deleted genes which reduces the size of the cDNA by 30% and allows its insertion into retroviral vectors while not compromising function.

Primary fibroblasts, myoblasts, hepatocytes and keratinocytes have been transduced with factor IX *ex vivo* using factor IX-containing retroviruses. To date, the only human gene therapy studies for haemophilia involved *ex vivo* transduction of skin fibroblasts (from two boys with moderate haemophilia B) with a retrovirus containing factor IX cDNA but as this proved successful in only one boy, this approach has not gained unequivocal support.

In vivo transfer methods have been explored. Factor IX-containing retrovirus constructs have been used to transfect dog hepatocytes via a portal vein catheter, but the procedure resulted in sustained but only low-level expression of transferred factor IX gene.

Modified adenoviruses have been used to transfect dog hepatocytes by directly perfusing the liver with virus through a portal vein catheter. An initial marked correction of factor IX levels was short-lived, probably because of immune-mediated clearance of the vector even when the experiments were performed with cyclosporin A.

Retroviral factor VIII transfer has resulted in expression of human factor VIII in many cell types, including human and murine skin fibroblasts, endothelial cells and murine bone marrow. Modified human skin fibroblasts persisted for up to two months after subcutaneous transplantation into immunodeficient mice but no factor VIII was detected in mouse plasma samples possibly because of very rapid clearance of human factor VIII from mouse plasma. Similarly, adenoviral systems in mice have resulted in short-lived production of human factor VIII.

Physical systems have employed a B-domainless factor VIII construct transfected into fibroblasts and myoblasts *ex vivo* using a receptor-mediated system. Transfected cells transplanted into mouse liver and spleen produced a significant but short-lived elevation in systemic factor VIII levels, but transplantation into muscle did not raise systemic levels, possibly because of factor VIII instability. Finally, it is crucial to identify which patient groups should first receive a potential gene therapy treatment in the clinical trial setting. Children with severe haemophilia will benefit most from gene therapy, but it may not be ethical to test such a novel and potentially unknown treatment on young children who are unable to give informed consent, especially when the potential for alterations to the immature germ line is great. Alternative groups are those with chronic blood-borne viral infection, such as HIV, whose long-term outlook is limited, but it is unlikely that such a group would be an acceptable first choice from a commercial viewpoint as the potential for predicted adverse events and risks of viral recombination events would be unacceptably high.

Bullet points:

- The aim of gene therapy in haemophilia is not to cure.
- Gene therapy aims to achieve a plasma level of 5-10 u/dL of the relevant coagulation factor, which protects against spontaneous bleeding and substantially improves lifestyle.
- The larger size of the factor VIII gene and its poorer stability as compared with factor IX means that most work has focused on factor IX cDNA.
- Recombinant viral vector techniques and physical gene delivery systems have produced short-term rises in factor levels in cellular and animal studies.
- Gene therapy for haemophilia B is unlikely to be available for clinical studies for at least five years - probably longer for haemophilia A.

STEM CELL TRANSPLANTATION

Since its first use in the 1960s in the treatment of severe combined immune deficiency states (SCID), stem cell transplantation (SCT) has been incorporated into the therapeutic regimens of many conditions including bone marrow failure and haematological malignancies (1970s), haemoglobinopathies (1980s), and solid tumours (1990s), and is likely to be used soon to treat autoimmune disorders. Approximately ten times more transplantation procedures were carried out in 1994 compared with 1984 (18,000 v 1,800), and the majority are now autologous procedures compared with allogeneic dominance ten years ago. Despite their widespread use, it remains imperative to evaluate allogeneic and autologous SCT in the context of other available therapies using controlled randomised clinical trials. The aims of pre-SCT conditioning using intensive chemotherapy +/- total body irradiation (TBI) are to remove disease, induce immunosuppression, and create space for stem cells in conditions associated with hypercellular marrows (chronic myeloid leukaemia, haemoglobinopathies). Stem cells can be collected from marrow, and from peripheral and umbilical cord blood, and sufficient repopulating cells must be given to the recipient to ensure engraftment success. At present, no reliable *in vivo* or *in vitro* assays are available to assess stem cell capacity, but assays using immunodeficient mice are promising. Post-SCT care aims to provide support prior to engraftment and prevent GVHD.

The main complications of SCT are seen more commonly with allogeneic rather than with autologous procedures, and are: i) immune-mediated - graft failure, GVHD, immunodeficiency states, ii) toxicity - chemotherapy +/- TBI-related; iii) disease recurrence; iv) late effects. Factors determining SCT outcome include age and disease stage at transplantation, and extent of HLA mismatch. Generally, better outcomes are obtained in young individuals who undergo SCT in the early stages of their disease once initial disease control has been achieved, and HLA mismatched SCT should be avoided except in very young children.

Approximately 25% of over 4,000 allogeneic procedures performed in Europe in 1996 were carried out for CML, and matched unrelated donor transplantation remains the treatment of choice for individuals < 50 years. Seven percent of allogeneic procedures were for severe aplastic anaemia, and this remains the preferred treatment if a sibling or matched unrelated donor is available and the recipient is < 40 years. Although 10% of procedures were for genetic disorders (SCID and thalassaemia), properly-conducted trials are needed to determine if allogeneic transplantation improves outcome. Allogeneic transplantation plays a beneficial role in ALL treatment and 900

procedures were performed for this condition. The benefit of its incorporation into AML therapy has yet to be determined by clinical trial evaluation but despite this, over 1,100 individuals underwent allogeneic transplantation for AML. Similarly, the benefits of over 6,500 autologous procedures performed for haematological conditions (39% NHL, 28% myeloma, 14% HD, 9% AML, 5% ALL, 4% CML) will only be determined after clinical trial data analysis.

Even in conditions where SCT is of proven benefit, its application is limited by lack of an HLA-identical related donor due to the generally smaller European family size. Around 60% of needy patients do not have an appropriate donor and so less suitable options must be considered, including cord blood bank searches and alternative therapeutic strategies. An unrelated donor search is only successful in 50% of cases, successful patients can wait more than a year before donor identification occurs, the donor pool for non-Caucasians is extremely small, and MUD procedures are generally less successful than matched related donor procedures, although in certain conditions an HLA well-matched unrelated transplant procedure can be beneficial i.e. CML in chronic or accelerated phase, primary refractory AML, secondary AML, high-risk MDS, and very high risk (CR 1, Ph +ve) and high risk (CR 2) ALL.

Cord blood transplantation (CBT) procedures are in their infancy. They offer definite advantages of rapid donor selection, acceptability to ethnic minority groups and a reduced risk of transmitted infection in the transplantation material. The use of naive lymphocytes should theoretically facilitate a greater degree of donor-recipient mismatch, but this benefit, if proven, may be counteracted by loss of lymphocyte-mediated graft-v-donor effect. As with SCT, it is imperative to ensure that adequate numbers of repopulating cells are transplanted, as survival to 40 months is almost doubled by administration of a high cell compared with a low cell dose (33% survival with low cell dose [below median] v 60% with high cell dose [above median]).

Autologous and allogeneic transplantations can cure. Survival is influenced by the extent of HLA matching, the recipient's age and disease stage. Alternative sources of allogeneic stem cells (other than cord blood and marrow) need further investigation. Transplantations using HLA haplo-matched family donors are being evaluated, but immune reconstitution already appears to be a major limiting factor. It remains imperative that prospective comparisons with non-transplantation therapy are carried out to determine the real value of transplantation procedures in the treatment of haematological conditions.

Bullet points:

- Marrow transplantation procedures have increased tenfold over the last decade.
- The majority of procedures are autologous, compared with previous allogeneic dominance.
- The role of autologous and allogeneic stem cell transplantation must be evaluated in the context of other available therapies using randomised controlled trials.
- The main early complications of stem cell transplantation occur with allogeneic procedures.
- They are: immune-mediated, conditioning-associated toxicity, and disease recurrence.
- Age and disease stage at transplantation, and extent of HLA mismatch are important determinants of stem cell transplantation outcome.

REFERENCES

- ¹ Bertina A, Koeleman RPC, Koster T *et al.* Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994; 369:64-7.
- ² Dahlback B. New molecular insights into the genetics of thrombophilia. Resistance to activated protein C caused by Arg 506 to Gln mutation in factor V as a pathogenic risk factor for venous thrombosis. *Thromb Haemost* 1995; 74:129-48.
- ³ Vandembroucke JP, Koster T, Brië E *et al.* Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *Lancet* 1994; 344:1453-7.
- ⁴ De Stefano V, Leone G, Mastrangelo S *et al.* Thrombosis during pregnancy and surgery in patients with congenital deficiency of antithrombin III, protein C, protein S. *Thromb Haemost* 1995; 74:793-4.
- ⁵ Finazzi G, Brancaccio V, Moia M *et al.* Natural history and risk factors for thrombosis in 360 patients with antiphospholipid antibodies: a four-year prospective study from the Italian Registry. *Am J Med* 1996; 100:530-6.
- ⁶ McColl MD, Ramsay JE, Tait RC *et al.* Risk factors for pregnancy associated venous thromboembolism. *Thromb Haemost* 1997; 78:1183-8.
- ⁷ US Department of Health and Human Services. Agency for Health Care Policy and Research. Acute Pain Management: operative or medical procedures and trauma. Rockville (MD): The Agency; 1993. Clinical Practice Guideline No 1. AHCPR Publication No. 920023:107.
- ⁸ ASCO. Update of recommendations for the use of hematopoietic colony-stimulating factors: evidence based clinical practice guidelines. *J Clin Oncol* 1996; 14(6):1957-60.
- ⁹ Byrne JL, Haynes AP, Russell NH. Use of haemopoietic growth factors: commentary on the ASCO ECOG guidelines. *Blood Reviews* 1997; 11:16-27.
- ¹⁰ Binet JL, Auquier A, Dighiero G *et al.* A new prognostic classification of chronic lymphocytic leukaemia derived from a multivariate survival analysis. *Cancer* 1981; 48:198-206.
- ¹¹ Pasi JK. Gene therapy for haemophilia. *Bailliere's Clinical Haematology* 1996; 9:305-17.