Advancing Laboratory Practice in Haematology VI 12–15 September 2001, Jersey, Channel Islands

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NHS Pathology services – the first 50 years and the next 50 years

Since its beginning, the National Health Service (NHS) has had a profound effect on the way pathology services are configured, staffed and delivered in the UK, and will continue to do so. One hundred and fifty years ago, there were no pathologists and no laboratories in hospitals. Medical schools employed museum curators who were also demonstrators of morbid anatomy. These were mostly young doctors working for a pittance while awaiting a chance to join the ranks of physicians or surgeons. They studied the nature of disease by postmortem examination and collected specimens for clinical teaching. Clinicians carried out simple chemical tests on urine and other body fluids in ward side rooms.

Things changed towards the end of the 19th century when serviceable microscopes became commonplace, staining techniques were developed and histopathology emerged from within morbid anatomy. Around this time the germ theory of disease became widely accepted and bacteriologists appeared on the scene. Larger medical schools had academic departments of pathology, but the evolution of hospital-based laboratories outside teaching centres was painfully slow. Many non-academic pathologists had to struggle for a living with a mixture of activities including hospital clinical pathology, public health work, coroners' postmortems and whatever other private work they could get. Where they could afford it, they employed unqualified laboratory assistants to help them; these assistants later became technicians.

By the 1920s, district hospital-based clinical pathology laboratories had appeared, where general pathologists provided services in morbid anatomy, histopathology, and microbiology. They also offered rudimentary tests in chemistry and haematology. The quality remained very uneven in different parts of the country until the establishment of the Emergency Medical Service (EMS) in anticipation of World War II. This resulted in urgent upgrading of hospitals throughout the country. It generated massive investment in pathology on a regional basis. It also provided pathologists and their technicians with secure salaries for the first time.

After the war, the EMS seamlessly moved into the NHS. Then, and only at this stage as state-funded laboratories became bigger and busier, the organizational fragmentation of pathology into separate disciplines began, first in teaching hospitals in the 1940s and 1950s, and later in district hospitals. The College of Pathologists, founded in 1964, sealed this process with its single-speciality membership examinations.

This pattern of professional development was possible because, unique to Britain, the NHS supported single-specialty pathology consultants in all its district hospitals without the restraint of any need for commercial viability. The legacy of the first 50 years of NHS-funded pathology is therefore that most district hospitals have separate and autonomous departments for the four main disciplines (and sometimes others, such as immunology). Latterly, a financial strain has begun to tell, because pathology services are mostly funded by NHS Trusts as a direct cost or overhead. Shrinking budgets have been paralleled by lack of capital investment and many laboratories are now poorly housed and equipped. Salaries for biomedical scientists have failed to remain competitive and there is a poor career structure, causing problems in recruitment and retention. Many laboratories are therefore under-staffed, under-resourced, poorly capitalized and using outdated working practices hobbled by lack of investment in communication technology.

So what will happen to UK pathology in the future? What is certainly needed is some serious re-examination of the highly compartmentalized management structure that still exists in many places. There also needs to be a review of how pathology is funded to make it more dependent on workload; a look at how the service is configured, where there is unnecessary duplication of expensive equipment; and consideration of the best deployment of senior professional staff. All this is not an argument for cost-cutting rationalization, or for carrying out all tests in an aircraft hangar in the East Midlands. However, we do need to examine better ways of meeting the demands of the service and this will involve putting some sacred cows at risk.

Larger, more expensive machines push us towards 24-h working to make them economic. They may not be justified in every hospital where transport times are short.

Cervical cytology screening is certainly something that could be centralized and should be, both for economic reasons and, more importantly, for improved quality. Histopathologists may be more efficient and achieve greater quality assurance if more of them work together, subspecialize and share technical facilities. Whether every hospital needs full-time resident consultant pathologists in every discipline needs to be dispassionately questioned; most probably do, but perhaps not all. There is a need for caution, because presently the quality of pathology in the UK is high, despite all the problems already described here. Furthermore, changes that are beneficial in one area may not be in another, owing to the different configuration of clinical services and the local geography. However, the political pressure for change coupled with the continuing forces of evolution will not allow pathology to stand still.

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Erythro-haematology and the red revolution

The red revolution is coming! Haematological focus for the last several decades has bypassed what has been seen to be the rather mundane and unexciting red cell. The introduction of automated cell counters which can characterize red cells in new and informative ways, the advent of recombinant cytokine therapies which can influence erythropoiesis and a growing realization that adequate oxygen delivery might actually be medically important as well as the over-riding concern of the patient are about to change all that.

At this stage, rather than review the advances that have taken place, it will be worthwhile to explore what is presently *not* known about erythropoiesis. The first parameter to consider, but not necessarily the most obvious, is the haemoglobin concentration. This is a measurement that everyone can make and everybody understands its significance. But do they? What does the haemoglobin concentration actually represent other than the local concentration of a red protein? Is it a reflection of the total red cell or haemoglobin mass? Is the concentration of haemoglobin in a peripheral vein of any relevance to that in a critical tissue capillary bed? Does it in anyway determine oxygen delivery to critical tissues? At the moment, we do not know the answers to any of these questions.

As far as red cell mass is concerned, while it can be, but is not always, accurately measured, we are not collectively clear about how the results should be expressed. It is obvious that the size of the individual will have a significant effect on the total mass of red cells but how to allow for that is a matter of taste. At the moment, the simplest way is to relate it to whole body weight but to recognize that, in particularly fatty individuals, this may be a poor reflection of the perfused lean body mass. There are other more sophisticated ways of doing this but none has been proven to be more or less effective at answering the simple question as to whether or not there are sufficient amounts of haemoglobin and red cells in the body.

Then there is the size of the red cells in the circulation. This is a difficult parameter, because the concept of red cell volume is fundamentally flawed. It is almost certain that red cells have different volumes in different parts of the circulation. Despite increasing sophistication of modern cell counters, the MCV on which much trust is placed is, in fact, a confidence trick. Cell counters are unable to measure the volume of the native cells and all of them must first 'sphere' the cell. The speed and degree to which this is carried out vary between different counters and will be greatly affected by other factors such as plasma osmolality. The much disregarded MCH, however, does not suffer from these problems but the mean figure does not in any way represent the possible heterogeneity of the cells in the circulation.

What determines the size of red cells is still very much a matter for speculation. The original Stohlman concept was that developing red cells continue to divide until their internal haemoglobin concentration reaches a critical level. This then suppresses further mitosis. At first (in the 1960s), this seemed to agree with the results produced by the new Coulter S counters, which showed that MCHC appeared to be confined within very narrow limits. The concept took a knock when it was realized that it was the machine producing the constant MCHC and that this artefact was not reproduced in other counting systems. It is now clear that red cells have a wide distribution of haemoglobin concentration, even in normal subjects.

At the moment, the best explanation of red cell size is that anything which interferes with DNA synthesis, and hence erythroblast mitosis, will result in fewer divisions in the marrow and larger red cells emerging. Conversely, anything which interferes with haemoglobin synthesis will result in red cells emerging with suboptimal amounts of haemoglobin. As these reticulocytes mature, the cell membrane and volume shrink to fit the reduced haemoglobin content. Whether this is true or not we cannot yet say.

Then there is the reticulocyte count. Leaving aside the continued confusion between the visual demi-semi-qualitative and the quantitative automated count, there is still a depth of residual confusion arising from previous

generations. The chief of these is the expression of the results as a proportion or percentage without realizing that this does not tell us about marrow productivity but simply about the proportion of young cells to old. Erythropoietic productivity itself is best assessed by the reticulocyte count, but even this assertion is based on minimal real data. Equally embedded is the myth that a diminished PCV calls for the reticulocyte count to be 'corrected'. This pernicious notion was founded on the assertion that reticulocyte maturation time was increased in anaemia as the result of the premature release of reticulocytes from the marrow. There is indeed an acute response to increased erythropoietin levels in which immature reticulocytes appear in the circulation but the balance is restored within days. The lifespan/maturation time of the reticulocyte is unrelated to the PCV.

What is really puzzling is why the haemoglobin concentration, red cell count and reticulocyte count are as stable as they are. Much of this is, of course, explained by the huge inertia that the red cell mass represents – new red cells are produced at roughly 1% per day, so that any change in mean values is going to take about 50 days to make an impact. However, given that the marrow can operate at up to 12 times the normal rate without undue exertion, perhaps we should think more of why it does not do that more often. What are the factors which prevent overproduction of red cells? The simple explanation based on the hypoxia/erythropoietin positive feedback concept is not longer tenable.

There is more unknown than known about red cells and this provides the prospect of years of happy exploration for those that have the time and inclination to do so. Long live the revolution.

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Cancer and anaemia

Incidence of anaemia in patients with cancer Anaemia is a common problem in patients with cancerous illnesses (Groopman & Itri, 1999). The frequency with which it occurs varies according to the tumour type and the treatment given (Table 1).

Causes of cancer-related anaemia Common causes of anaemia in patients with cancer are:

- blood loss;
- iron or folate deficiency;
- bleeding;
- haemolysis;
- bone marrow infiltration by tumour;
- secondary to treatment with chemo- and/or radiotherapy.

However, in most patients the only cause will be the cancer itself (Means, 1999). This cancer-related anaemia is similar to that found in certain patients with chronic inflammatory disorders such as rheumatoid arthritis or chronic infectious conditions, and is usually described as the anaemia of chronic disease (AOCD).

Haematological findings in the anaemia of chronic disease Patients with the AOCD have elevated serum levels of several cytokines, including IL-1, TNF-alpha, gamma interferon and beta interferon. Sub-normal levels of erythropoietin are found in most patients with cancer (Miller, 1992).

The raised cytokines and the low erythropoietin level result in reduced erythropoiesis and shortened red cell survival (Means, 1999).

A common feature of the AOCD is impaired iron utilization by the developing red cells, which probably also contributes to the anaemia.

Symptoms of anaemia and its treatment For many years anaemia in patients with cancer was regarded as an inevitable effect of the disease and/or its treatment and it was considered that it usually required no treatment.

Until the last decade, blood transfusion was the only reliable treatment for the anaemia but transfusions are still rather sparingly used (Barrett-Lee *et al.*, 2000). Blood transfusion reliably increases haemoglobin concentration but this benefit is often short-lived and further blood transfusions may be required. The patient's sense of well-being seesaws with the rise and fall in haemoglobin.

Recent data from large community-based studies (Glaspy *et al.*, 1997; Demetri *et al.*, 1998) and from randomized trials (Osterborg *et al.*, 1996; Littlewood *et al.*, 2001) demonstrate that treatment of anaemia with erythropoietin improves the haemoglobin concentration, decreases transfusion need and improves quality of life. One study, in over 4000 patients, has shown that the maximal improvement in quality of life occurs as the haemoglobin rises from 11.0 to 12.0 g/dl. and the improvement in quality of life occurs up to a haemoglobin concentration of around 14.0 g/dl (Cleeland *et al.*, 1999).

Erythropoietin produces a sustained rise in haemoglobin but a response (Hb rise > 2.0 g/dl) is achieved in only 50–60% of patients. Predictors of a good response include low baseline serum erythropoietin levels, a rise in haemoglobin of > 0.5 g/dl within 2 weeks of starting treatment

Table 1

Malignancy	% of patients	Hb level
Myeloma (San Miguel *et al*., 1995)	50% on presentation. Majority during initial chemotherapy	< 10.5 g/dl
Non-Hodgkin's lymphoma (Coiffier 1999)	40% at diagnosis. 70% after 6 cycles of chemotherapy	< 12.0 g/dl
Solid tumours ($n = 2719$ various types) (Barrett-Lee *et al*., 2000)	38% during treatment. (33% required at least one blood transfusion)	< 11.0 g/dl

(Ludwig *et al*., 1994) and a normal platelet count (Osterborg *et al*., 1996). Most responders to erythropoietin do so within a month of commencing treatment but it can take up to 8 weeks for the maximum response to be achieved.

The haemoglobin response to erythropoietin in patients with myeloid disorders such as myelodysplasia is substantially less good than for patients with non-myeloid haematological malignancy or solid tumours (Hellstrom-Lindberg, 1995).

Anaemia and survival Anaemia is an adverse prognostic factor for patients with lymphoma, myeloma, chronic lymphatic leukaemia and in some patients with solid tumours.

There are a number of possible mechanisms by which a low Hb concentration may impair survival:

- impairing tumour oxygenation, thereby reducing the effectiveness of chemotherapy and radiotherapy and increasing the tumour's malignant potential;
- indirect mechanisms resulting from a decrease in patients' QOL; and
- decreasing the amount of treatment delivered to the patient possibly because of the above.

Recent data suggest that correcting anaemia with erythropoietin results in an improvement in patient survival (Littlewood *et al*., 2001) but this finding requires confirmation.

Conclusions Anaemia has long been considered an unimportant fact of life for patients with malignant disease. A substantial literature has demonstrated that anaemia impairs quality of life and adversely impacts on survival. Recent studies have shown that correcting the anaemia with erythropoietin improves the haemoglobin concentration, decreases transfusion need and improves quality of life. Very recent data have also suggested that correcting anaemia might improve the patients' life expectancy but this finding needs to be confirmed.

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B12 folate haematinic assay

Like many other laboratory assays, requests for estimation of serum cobalamin and folate have increased markedly in recent years. In the past, the main indication for

performing the assays was an unexplained macrocytosis of red cells,or morphological changes on a peripheral film suggestive of megaloblastosis. Guidelines from the British Committee for Standards in Haematology (BCSH General Haematology Task Force, 1994) broadened the consideration of the need for testing to patients who have clinical conditions, including dementia, which may be associated with cobalamin or folate deficiency, independent of peripheral blood morphological changes. The main purpose of the laboratory assays is to answer a clinical question about the possibility of a vitamin deficiency, and investigation must be followed by interpretation of the results, with appropriate action leading to the optimum patient outcome. This presentation will use data collected in a large teaching hospital by medical students during final year laboratory and clinical audit projects that were carried out to assess the appropriateness of the indications for requests for B12 and folate assays. In addition, interpretation of the results, and how they affected patient management will be considered.

In a prospective study (Huntly *et al.*, 1997), 1081 consecutive requests were divided into four groups according to the red cell MCV and the clinical information on the request form. Tests were regarded as: (1) *Indicated* (macrocytosis or morphological features of megaloblastosis – 384 samples; (2) *Uncertain* (no macrocytosis or features of megaloblastosis, but a clinical condition which could be associated with cobalamin or folate deficiency – 282 samples); (3) *Not indicated* (no macrocytosis or features of megaloblastosis or clinical condition associated with cobalamin or folate deficiency – 320 samples); (4) *Unknown* (no macrocytosis or features of megaloblastosis, and no or insufficient clinical information – 95 samples). Abnormally low results in each group were: (1) Indicated (21.1%); *Uncertain* (5.3%); *Not indicated* (5.9%); *Unknown* (8.8%). A higher yield of abnormal results was thus obtained in those with haematological changes. However, there was no difference in the percentage of abnormal results in the 3 latter groups, and thus no rationale for restricting assay of samples without haematological changes to patients with a clinical condition which may be associated with cobalamin or folate deficiency.

The interpretation of borderline or low serum cobalamin or serum and red cell folate concentrations in populations extended beyond those with clear haematological abnormalities may be difficult. A therapeutic trial of replacement therapy may still be the clearest way to establish a deficiency in an individual patient. However, benefits of treatment in the elderly with neurological abnormalities remain uncertain (Matthews, 1995). Metabolic changes (changes in methymalonic acid and homocysteine) may be a marker of more subtle deficiencies (Stabler *et al.*, 1990), some of which may have pathological consequences (e.g. poor folate status and fetal neural tube defects, or vascular damage related to higher serum homocysteine concentrations) (Wickramasinghe, 1999).

The response of clinicians to low results for serum cobalamin and folate was assessed by review of the case notes of 16 patients: only 2 had been investigated and managed appropriately, and in the majority the abnormal result appeared simply to have been filed. Uncertainty about how to interpret borderline or low results may have contributed to this poor outcome in this small sample. The Medicines Monitoring Unit (MEMO) in Tayside enables a record linkage between laboratory data and all community prescriptions, allowing examination of clinicians' treatment in response to the results of cobalamin and folate assays in a larger group of patients (Gelly *et al.*, 1997). Low cobalamin or folate concentrations were detected in 47/740 patients, but only 24 (51.1%) received a prescription for cobalamin or folate. Of 99 patients with a borderline value, only 27 (27.3%) received a prescription. Conversely, of the 594 patients with normal or high values, 60 (10.1%) received a prescription. These results indicate a distressing lack of concordance between the results of the laboratory assays and the clinical outcome.

The normal physiology of cobalamin and folate, and its disturbances in deficiency states, leads to difficulties in interpretation of serum cobalamin, and serum and red cell folate concentrations. However, these difficulties appear to be compounded by a wasteful approach to using these investigations and an irrational response to the data obtained. Our guidelines need to be extended to encompass the outcome for the patient.

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Vitamin B_{12} and cognition: mind over marrow?

In 1989 a 59-year-old steelworker was referred by his general practitioner to the local psychiatric out-patient department with a 9-year history of progressive memory loss. There was a strong family history of dementia; his father had died of a dementing illness aged 74 and four of his siblings had died before the age of 60 years with presenile dementia. There was no relevant medical history other than a myocardial infarction 11 years previously. On examination he was disorientated in time and place scoring only 14 out of 30 on Mini Mental State examination. There was little spontaneous speech and there were marked word-finding difficulties. Cranial nerve examination was normal. His gait was slightly stooped but with normal arm swing and no extrapyramidal signs. A pout reflex was elicited. He had myoclonus in his arms and legs but his limbs were otherwise normal with regard to power, tone, reflexes and sensation.

A CT brain scan demonstrated cerebral atrophy in excess of that expected for his age. Serum folic acid, thyroid function test and treponemal serology were normal, but his mean cell volume was raised at 102 femtolitres (fl) and his γ glutamyl transferase was also elevated consistent with his daily consumption of home brewed beer. Serum vitamin B_{12} was markedly reduced at < 50 ng/l (normal 200–1000 ng/l) and he had been commenced on replacement therapy. A Schilling test was normal.

Because of the strong and unusual family history of dementia, this patient's family were referred for genetic studies and were subsequently one of the first families in which a genetic mutation was found in the amyloid precursor protein gene (APP 717 Val $\rightarrow$ Gly) (Kennedy *et al.*, 1993).

Whilst compiling and summarizing records, other affected members of this family were also noted to have low values of Vitamin B_{12} and folate. A further study of this family found lower serum Vitamin B_{12} in affected individuals compared with unaffected siblings (McCaddon & Kelly, 1994).

It was hypothesized that such deficiencies could contribute to cognitive decline (McCaddon & Kelly, 1992). The relevance of this hypothesis for late-onset dementia was investigated by measuring homocysteine in patients with senile dementia of Alzheimer-type. Homocysteine is a marker of subtle B vitamin deficiencies. Patients had higher serum levels of homocysteine than age-sex matched controls (McCaddon *et al.*, 1998). The Oxford University-based OPTIMA project subsequently confirmed this observation (Clarke *et al.*, 1998). Other studies have since reinforced these observations, suggesting that elevated homocysteine might be an early marker for cognitive decline in the elderly (Lehmann *et al.*, 1999).

These subtle deficiencies appear to be independent of nutritional status, and suggestive of aberrant B_{12} metabolism in Alzheimer's disease. This was investigated further, and patients were found to have proportionately more inactive forms of the vitamin in blood than healthy controls (McCaddon *et al.*, 2001a).

These early studies culminated in a recently completed project funded by the Wales Office of Research and Development entitled 'COBALZ II'. COBALZ II has verified the genetic basis underlying transcobalamin isotypes, confirmed that dementia patients have subtle B_{12} and folate deficiencies in the absence of anaemia, and shown that the relationship between homocysteine and Alzheimer's disease is independent of clinically diagnosed vascular disease.

We have also completed a five-year longitudinal study showing that homocysteine is an independent predictor of cognitive decline in healthy elderly (McCaddon *et al.*, 2001b). Higher blood levels of homocysteine are associated with a more rapid decline in mental faculties over a 5-year period. This suggests that elevated homocysteine contributes to, rather than results from, impaired cognitive function.

The original 'cobalaminergic' hypothesis also predicted that Alzheimer's disease patients should have low levels of Vitamin B_{12} on the carrier protein transcobalamin. The OPTIMA team have now confirmed this, and shown that holo-transcobalamin appears to be a better marker than total B_{12} for the assessment of vitamin B_{12} status in Alzheimer's disease (Smith *et al.*, 2001). This observation further strengthens the hypothesis that Vitamin B_{12} metabolism, as well as folate, is closely associated with the development of the disease.

There remain many unanswered questions regarding the relationship between these subtle metabolic deficiencies and Alzheimer's disease. Can a reduction in serum homocysteine levels prevent or delay cognitive decline? Is holo-transcobalamin a better marker of B_{12} status in neuropsychiatric patients? Do physiological forms of Vitamin B_{12} differ in patients vs. controls? Is there a transcobalamin receptor polymorphism that might account for these observed relationships between B_{12} and Alzheimer's disease?

Other research teams are currently addressing some of these important questions. Our own research objective continues to focus on the role of Vitamin B_{12} in the pathogenesis of Alzheimer's disease and related dementias. The primary motive for this research was the observation that members of the index family affected by an amyloid precursor protein gene mutation appear to develop a B_{12} deficiency in association with their dementia. The reason for an association between amyloid processing and B_{12} deficiency remains elusive, but a recent hypothesis offers one possible explanation.

It is generally considered that homocysteine potentiates oxidative injury in vascular disease and Alzheimer's disease. However, it is also possible that moderate elevations of serum homocysteine in dementia arise as a *consequence* of cerebral oxidative stress, resulting in a harmful feed-forward cascade.

This particular hypothesis explains several puzzling features concerning Vitamin B_{12} and Alzheimer's disease such as the absence of haematological abnormalities and the presence of inactive B_{12} analogues in these patients. However, it requires evaluation both *in vitro*, using suitable cell or animal models, and *in vivo*, by assaying cobalamin analogues and markers of oxidation in cerebrospinal fluid of patients with Alzheimer's disease. Longitudinal studies might also assist in unravelling the temporal relationship between oxidative stress and disturbed B_{12} metabolism. We plan to use a combination of these approaches further investigate this particular hypothesis.

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Airline travel and venous thrombosis – is there a risk?

Recently there has been considerable controversy, particularly in the popular press, about whether long-haul economy airline travel increases the risk of venous thromboembolism. Every year the number of passengers travelling over long distances by air increases and the distance between economy class seats (the so-called 'pitch') remains very limited. The standard economy seats being somewhere between 70 and 85 cm apart. The potential risks of air travel arise from sitting for prolonged periods in a confined space, pressure on the back of the calves, dehydration often heightened by drinking excess free alcohol, poor air quality and hypoxia. It would seem that the passengers most at risk are those who are grossly overweight, are very tall with very long legs and or who have had previous problems with varicose veins. Obviously, passengers will also have a variety of acquired and inherited risk factors for the development of venous thrombosis, and it is now accepted that about 10% of the adult population will have a specific acquired or inherited defect in the haemostatic mechanism that to some extent increases the risk of development of venous thromboembolism. Physicians and haematologists working close to major airports are now frequently seeing individual cases presenting with thromboembolic problems which may be related to air travel. Results of retrospective clinical series suggest that up to 20% of patients presenting with thromboembolism have undertaken recent air travel. However, the true frequency of the problem remains unknown and is still controversial and, recently, a Dutch group looked at all forms of travel in the previous 4 weeks in patients presenting with acute deep vein thrombosis (DVT). They concluded that travelling times of more than 5 h were not associated with an increased risk of DVT (Kraaijenhagen *et al.*, 2000). It must be remembered that episodes of DVT can occur without any symptoms, although a few of such patients may go on

to have a clinically detectable and potentially fatal pulmonary embolism.

We have conducted a prospective randomized study in which we studied 200 volunteer long-haul passengers undertaking two legs of at least 8 h each and then returning to the UK. All subjects had duplex ultrasonography of the lower limbs before and up to 48 h after their return from travel. Passengers were randomly allocated to one of two groups (100 in each); one wearing class I below-knee graduated elastic compression stockings, while the others did not. Blood samples were tested for the Factor V Leiden (FVL) and prothrombin gene mutations (PGMs) and anticardiolipin antibodies. A sensitive D-dimer ELISA assay was performed before and after return from travel. Volunteers with a previous history of thrombosis or malignancy were excluded. Twelve passengers developed asymptomatic calf DVT – none of these was wearing elastic compression stockings. Of the 100 volunteers wearing stockings, none developed DVT but four developed superficial thrombophlebitis possibly associated with local superficial varicose veins. Some 7% of the volunteers had either heterozygous FVL or prothrombotic gene defects (11 with FVL and four with PGM). Two of the passengers with DVT and FVL and one of the passengers with superficial thrombophlebitis were doubly heterozygous for FVL and PGM. No passenger with a DVT developed an elevated D-dimer level after travel. Asymptomatic DVT occurred in 12% of 100 volunteers who did not wear stockings, whereas the simple measure of wearing compression stockings provided effective protection to prevent the development of DVT (odds ratio, 0.04; 95% CI, 0.02–0.95) (Scurr *et al.*, 2001).

Finally, one has to consider how to advise patients with a previous history of thrombosis or those who are known to have a specific haemostatic defect, particularly the FVL mutation, prior to long-haul travel. In the case of passengers who are not taking long-term oral anticoagulant therapy with Warfarin, I would recommend that these high-risk individuals should have a prophylactic subcutaneous injection of a low molecular weight Heparin preparation about 2 h before flying; in other words, when leaving their hotel or home to travel to the airport. In addition, I would advise all these individuals also to wear elasticated below-knee grade I compression stockings, ensuring that there is between 20 and 30 mm of pressure on the ankle. Obviously, patients who are already receiving long-term Warfarin and/or Aspirin should continue to do so. Further studies are needed to define precisely the exact approach in various passenger groups and, in conjunction with the World Health Organization, we are embarking on a multicentre follow-up study to try to clarify further the incidence, preventative measures and risks associated with long-haul flying.

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Blood clotting and haemostasis: what should we measure?

Conventional laboratory investigations of blood coagulation have remained essentially unchanged for many decades. The introduction of automated techniques coupled with the development of thorough quality assurance programmes have led to improvements in reproducibility and reliability, but the principles of the methods related to clotting tests on blood and plasma have not been modified. More recently, however, considerable attention has focused on the role of the vascular endothelium and the loss of endothelial integrity in the pathogenesis of haemostatic disorders. Methods have been devised especially to evaluate endothelial disturbances *in vivo,* and novel techniques have been developed specifically to assess inappropriate activation of the clotting mechanism. In addition, assays have been proposed to examine the association between inflammatory responses and abnormal haemostasis. The relative importance of these tests has not been firmly established, however, and the current presentation discusses the results of a number of diverse studies designed to explore their use in routine clinical practice.

The first investigation was undertaken to evaluate the use of commercially available ELISA for von Willebrand Factor (VWF), thrombomodulin (TM) and soluble adhesion molecules (ICAM-1, VCAM-1, E-selectin and P-selectin) in the routine management of patients with vasculitis. A range of patients were recruited over a period of approximately 12 months from regular hospital clinic visits. Normal control volunteers were also recruited over this time. Venous blood samples were obtained initially at diagnosis or when symptoms of active disease were notably evident. Repeat samples were obtained at intervals over a period of up to 36 months during and after therapy. The mean results of initial assays within the different patient groups demonstrated that in general, levels of circulating VWF, TM and ICAM-1 were significantly elevated. Similarly, the mean VCAM-1 and E-selectin assays were significantly raised in systemic lupus

erythematosus (SLE), giant cell arteritis (GCA) and glomerular nephritris (GN). VCAM-1 tended to be high in systemic sclerosis (SSc). P-selectin tended to be significantly higher in some patients, especially in rheumatoid arthritis (RA), although a very wide normal range was observed for this assay. There was a considerable overlap, however, between the assays on patients and those on normal controls and the measurements of different assays between groups poorly correlated with each other.

On repeated blood samples obtained after treatment, the levels of the different analytes tended to be higher in patients with the most severe clinical symptoms and in a number of cases the levels decreased after effective therapy. The results were not straightforward, however, and did not consistently reflect disease activity. In many instances, assays were higher after treatment than before therapy. No correlation was observed between any of the ELISA results and other determinants of disease activity such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Similarly, we have recently demonstrated that levels of VWFAg, TM and soluble E-selectin did not reflect the improvement in endothelial function (assessed by flow-mediated dilatation, FMD) after short-term (6 weeks) high dose folic acid therapy (5 mg daily) in patients with stable, confirmed coronary artery disease.

We have also utilized these assays to investigate the relationship between pre-existing disturbances in haemostasis and the risk of thrombotic and embolic events after cardiac surgery. We conducted a longitudinal study to correlate haematological, biochemical and microbiological data with subsequent clinical endpoints in 375 consecutive patients undergoing heart valve replacement. The haematological data demonstrated that there were marked differences between patients, with many abnormal values preoperatively, not explicable by age-related variation or the presence of arterial disease. In addition, very high levels of inflammatory markers (TNF-alpha and IL-6) were observed in a high proportion of patients, and the incidence of subsequent clinical complications appeared to correlate with evidence of pre-existing specific infection.

In addition, we have examined the effects of standard intermittent pneumatic compression (IPC) on these markers of blood coagulation and fibrinolysis. IPC provides an effective means of prophylaxis against postsurgical, deep venous thrombosis (DVT), but, although many studies have demonstrated consistent changes in haemodynamic parameters, the results are far from conclusive regarding systemic haemostasis. Our preliminary investigations confirmed that the method of blood collection markedly influenced results. In particular, tissue plasminogen activator activity (tPA) and plasminogen activator inhibitor activity (PAI-1) were raised *per se* in samples obtained from in-dwelling catheters, and levels of thrombin-antithrombin complex (TAT), prothrombin fragment 1.2 (PF1 + 2) and D-dimer were significantly elevated in samples obtained after difficult venepunture. In control experiments, without the use of compression, VWFAg was significantly lower and tPA was significantly raised after 120 min. In contrast, IPC in the same individuals led to highly significant, sequential falls in factor VIIa (FVIIa), associated with transient reductions in TAT and PF1 + 2 and a tendency to increased levels of tissue factor pathway inhibitor (TFPI). In addition, significantly higher levels of urokinase-plasminogen activator activity (scUPA) were seen using a chromogenic method but these changes were not evident using an ELISA.

Finally, we have re-investigated a previously reported kindred with an isolated abnormality of platelet procoagulant activity, using recently available methods for assessing platelet function. Flow cytometry (FCM) demonstrated that the reduced platelet prothrombinase activity was associated with impaired exposure of platelet phosphotidylserine (PS) together with defective release of platelet microvesicles, thus identifying a new case of Scott Syndrome. The findings clearly illustrated the potential for flow cytometry in assessing platelet and other cellular interactions. Studies of this nature could be rewarding in examining haemostasis in clinically demanding pathologies such as life-threatening sepsis, ischaemic heart disease and cancer.

Overall, our results have highlighted the importance of interrelated mechanisms in the pathogenesis of haemostatic disorders. Measurements of soluble adhesion molecules in plasma might provide clues in general but appear not to be helpful for individual diagnostic purposes in a routine clinical setting. Sensitive analysis of the primary triggers of blood coagulation and fibrinolysis might be informative but preanalytical variables require careful attention. Flow cytometry and assays of inflammatory responses could provide informative data. Our findings tend to cut across conventional disciplines and could provide challenging strategies for future diagnosis and therapy.

Further reading

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Advances in molecular haemostasis

Our understanding of the molecular basis of haemostatic defects is being advanced by the use of rapid techniques for mutation screening and DNA sequencing which are now being applied to many different genes.

The UK Haemophilia Centre Doctors Organization has recently recommended that the mutation responsible for haemophilia be sought in all patients at diagnosis. Several laboratories are now in a position to offer this service. Occasionally, this is performed by direct sequencing of the coding region of the gene, as described recently (Costa *et al.*, 2000; Vidal *et al.*, 2001). However, most laboratories still find this an expensive alternative and employ a mutation screening strategy prior to DNA sequencing, such as conformation sensitive gel electrophoresis (CSGE) (Williams *et al.*, 1998) or denaturing high performance liquid chromatography (DHPLC) (Oldenburg *et al.*, 2001). These techniques are both based on heteroduplex analysis, DHPLC has the advantages of being semi-automated and highly efficient at identifying sequence alterations and is being introduced into a large number of diagnostic and research laboratories to enhance mutation screening capabilities.

The common thrombophillic mutations factor V Leiden and prothrombin G20210A are analysed by several different methods in different laboratories. Rapid analysis can be achieved by multiplex PCR of both mutations in one tube and by DHPLC, 5′ nuclease assay (TaqMan) or heteroduplex analysis. These techniques are also being applied to the rapid analysis of single nucleotide polymorphisms in several other genes that may influence thrombotic tendancy (Andersson *et al.*, 2001). Rarer thrombophillic mutations in protein C, S and antithrombin genes are, like those in the factor VIII and IX genes, being sought by direct DNA sequencing and by rapid mutation screening techniques.

It is envisaged that, as mutation screening and sequencing become more widely available, our knowledge and understanding of the molecular basis of haemostatic disorders will be greatly enhanced.

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Waveform analysis of clotting test optical profiles*

Recent advances in the analysis of optical profiles obtained during the routine performance of the prothrombin time (PT) and activated partial thromboplastin time (aPTT) have led to the realization that there is more information from a clotting assay than just the clotting time. Originally, these assays were conducted manually by adding plasma, an activating agent and calcium chloride into a warmed test-tube and visually monitored to provide only single items of information, i.e. clotting times. However, in the hands of an experienced observer, a qualitative, albeit subjective, assessment of fibrin polymerization could be made. Thus, important additional information could be obtained. As these tests were automated, to facilitate and standardize their performance, this advantage was lost. Automated devices based on mechanical principles to measure the clotting time clearly could not accommodate the visual attributes of the manual method. While photo-optical systems had this potential, first generation systems were designed to report only clotting times. However, advancements in both photometer design and the optical signal processing at bioMerieux have led to the recovery of information that can be graphically represented as an optical profile or waveform constructed by plotting changes in light transmittance as a function of time. This provides access to qualitative information that was lost when the manual method was replaced. Moreover, the information available is now both objective and quantifiable. Coagulation is a dynamic

*US Patent 5,749,976.

process and the complete analysis of the optical profile can yield quantitative information on the time, acceleration, rate and magnitude of the process leading to fibrin polymerization.

The ability to perform these procedures has been incorporated exclusively in the MDA® series of coagulation analysers (bioMerieux). The photometer in these analysers is designed (1) to measure relative changes in transmittance instead of absorbance; (2) to gather the signal over the entire course of clot formation; (3) to normalize light levels on a per test basis thus adjusting for variations in specimen optical density; and (4) to employ kinetic algorithms for clot detection. The instrument software also incorporates analytical routines that define, on-line, a set of nine parameters that characterizes the optical data obtained during the performance of the aPTT and PT assays (Braun *et al.*, 1997). These parameters are defined by dividing the waveform obtained into three sectors: the precoagulation, coagulation and postcoagulation phases. Events, with respect to time, acceleration, rate and magnitude, may then be calculated for each of these phases. The software has also been designed to facilitate the export and analysis of waveform data for both routine and research settings. Preliminary studies performed in house by bioMerieux demonstrated that it was possible to predict the presence of heparin or factor deficiencies and provide an estimate of fibrinogen concentration from both PT and aPTT assays. (Givens *et al.*, 1996, 1997). Subsequently, independent investigators have confirmed the utility of waveform analysis in a number of different clinical settings.

A change in the precoagulation phase (Slope_1) of the aPTT waveform, resulting in what is referred to as a 'biphasic waveform', has been shown to be an indicator of the presence of sepsis and disseminated intravascular coagulation (DIC) (Downey *et al.*, 1998) and to be highly correlated to patient outcomes in critical care units. As the analysis is performed on-line, detection of this abnormality can be automatically flagged (A2 Flag) to alert the laboratory and, in turn, the attending physician to the emergence of an important and potentially life threatening medical complication. Slope_1 analysis of the PT shows great promise in the detection of Lupus Anticoagulants (Ortel T *et al.*, unpublished). Finally, complete waveform analysis of the aPTT appears to provide additional discriminatory power over clotting time and/or one-stage factor assay in defining the clinical severity of the classical haemophilias. Moreover, it appears to provide a more accurate measurement of Factor VIII/IX levels below 1.0 μ/dl (Shima M *et al.*, unpublished). Thus, waveform analysis has the potential to streamline diagnostic test procedures by providing additional information during the performance of a single test. In turn, this provides the possibility for improving patient outcomes, by expediting and optimizing therapeutic interventions, and decreasing overall health care costs.

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Transmittance waveform analysis of routine coagulation tests is a sensitive and specific method for diagnosing non-overt disseminated intravascular coagulation

We have previously shown that the transmittance waveform profile on the routine coagulation test of the activated partial thromboplastin time (aPTT) can be characteristically biphasic in patients with disseminated intravascular coagulation (DIC). Two particular features of note, were (a) the increasing positive predictive value for DIC with increasing abnormality in this profile, and (b) waveform changes often preceded those in the more conventional parameters used for diagnosing DIC.

To examine if a single analysis of the aPTT waveform could assign risk in terms of DIC development, the analysis was performed within the first hour of admission on 1187 consecutive patients admitted to the intensive therapy unit (ITU) over a 24-month period. The degree of change causing the biphasic waveform (BPW) was quantified through the light transmittance level at 18 s (TL18) into the aPTT reaction on the MDA 180® coagulation analyser (bioMerieux) and assessed against the development of DIC in these patients. DIC was defined using the Japanese Ministry of Health and Welfare 1988 criteria. In parallel, mortality estimates and the diagnosis of sepsis were determined in all patients.

Three hundred and forty-six patients were found to have a BPW on admission to the ITU and 169 of these patients developed overt DIC. This was out of an overall 362 who had DIC. A logistic regression model fitted the data satisfactorily and a stepwise increase in the likelihood of DIC was directly correlated with the degree of TL18 change. The DIC fraction was 0.21 in absence of BPW, which increased to 0.76 with a 25% reduction in TL18. In addition, detection of the BPW preceded the diagnosis of DIC by 18 h in 46% of the patients. Mortality

in this subgroup, where the first BPW detected DIC at a non-overt stage, was 58% and little different from those where the BPW was concurrent with overt DIC (61%). Overall, 122 of the DIC cases were due to sepsis with 54 (44%) developing the first BPW in the non-overt/pre-DIC stage.

Waveform analysis within the first hour of ITU admission is a single, simple and rapid method of identifying the risk of developing DIC. It is sensitive to the early and non-overt stage of the DIC process and provides a valuable tool in triaging ITU patients so that prompt and appropriate treatment can be targeted, especially for patients with sepsis.

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Modernization of pathology in Manchester

The city of Manchester is served by two teaching hospital trusts (Central and South Manchester) and North Manchester General Hospital. Within the city and the Greater Manchester conurbation, the configuration of health services is currently undergoing a period of unprecedented change. As a consequence of Private Finance Initiative (PFI) developments at South Manchester University Hospitals Trusts (SMUHT), patients will transfer to Central Manchester. At Central Manchester and Manchester Children's University Hospitals NHS Trust, a £250 million PFI development will facilitate the transfer of children's services into 'purpose designed' accommodation in conjunction with the re-provision of adult clinical services. In parallel with these transfers other changes are developing in relation to the reconfiguration of clinical services, e.g. rationalization of renal services and neurosciences.

Concurrent with the developments already mentioned, there have been a number of reviews of pathology services in Manchester culminating in the review carried out by Touche-Deloitte in 1996. This particular review recommended pathology rationalization and a reduction (saving) in funding of about £1 million across the city. This financially based review was poorly received by the professional laboratory medicine community. Manchester Health Authority subsequently commissioned a professional/peer review.

This review, carried out by Professor Bellingham (past president RCPath) and reported in 1997, made specific recommendations regarding the configuration of pathology services in Manchester:

- the transfer of cytology services and specialist services to Central Manchester Healthcare Trust (CMHT);
- the establishment of appropriate Microbiology Services at SMUHT;
- the development of a Unified Microbiology Service involving close working relationships between CMHT and the Public Health Laboratory Service (PHLS);
- transfer of specialist services to Central Manchester;
- GPs to use a provider unit of their choice.

Project 2000 was initiated in autumn 1999 in order to initiate and deliver aspects of the Bellingham recommendations.

The project has been developed and progressed through the coordinated efforts of the Laboratory Medicine Professional staff including medical, biomedical and clinical scientists working together.

Arrangements were established in March 2000 to organize and manage the progression of the project objectives. These arrangements involve a Chief Executive Officers/Regional Office, Project Steering Group chaired by the Regional Office and a Project Working Group chaired by Manchester Health Authority. Supported by the NHS Executive (North-west) and Manchester Health Authority, the Project 2000 initiative has developed as a partnership between the following organizations:

- Central Manchester and Manchester Children's University Hospitals NHS Trust (CMMCUH);
- Christie Hospitals Trust;
- Manchester University;
- PHLS;
- SMUHT.

The project involves the design and construction of an extension to the Clinical Sciences Building at CMMCUH based at Manchester Royal Infirmary and the transfer of staff and services.

Key features of the project

- First steps in partnership working.
- Transfer and integration of Cytology Laboratory Services.
- Enhancement of Manchester Cytology Training Centre.
- Transfer and integration of Manchester PHLS.
- Establishment of Unified Microbiology Service.
- First steps in the establishment of a managed clinical network.
- Delivers aspects of the 'Bellingham' recommendations.
- Facilitates transfer of clinical work upon closure of Withington Hospital.

- Establishment of microbiology laboratory facilities at Wythenshawe Hospital.
- Further development of Autolab facilities at Central Manchester.
- Development of molecular biology facilities at Central Manchester.
- Efficiency savings.

Cytology The development will involve the transfer of 50 staff, and will create one of the largest cytology centres in Europe, which will be fully integrated with gynaecological pathology, and the clinical departments of gynaecology and oncology at St Mary's Hospital. The modern laboratories and equipment will enhance the prospects of profitable research into new screening techniques. The Manchester Cytology Training Centre is to be housed in purpose-built accommodation and will continue to offer high-calibre training to staff throughout the North-west region.

Interim management arrangements for the Cytology Service were introduced in advance of physical merger (June 2000). A combined management structure is in place reporting to a cytology management board consisting of representatives of both Trusts and Manchester Health Authority.

Manchester PHLS This Involves the transfer of > 100 staff. Following integration with staff and facilities at Central Manchester a 'unified service' for the provision of microbiology services will be developed. This new service will provide comprehensive microbiology services to Central Manchester, SMUHT and Christie Trust, and specialist services to a wide range of Trusts across the North-west region and beyond. In addition to the development at Central Manchester, microbiology (bacteriology) laboratory facilities will be established at Wythenshawe hospital.

Management arrangements for the Unified Microbiology Service were agreed in autumn 2000. The arrangement will be established following agreement of a 'Memorandum of Understanding' between Central Manchester and PHLS, and prior to the 'physical' merger of the two organizations.

Autolab facilities Further development of the joint 24 h biochemistry\ haematology automated laboratories (Autolab). This non-stop pathology facility will continue to develop enhanced automation (pre-analytics and robotics) capabilities and will facilitate technology transfer with realizable benefits to service provision. Specifically, the development will facilitate the transfer of GP (community) pathology services and in-patient services from Withington.

The automation and Autolab development is 'evidence based', following detailed studies of laboratory services in 10 of the top 20 hospitals of the USA, Japan and Europe. At least five targeted laboratory automation (TLA) systems are fully operational in the USA, and there are a number in Japan as well as recent installations in Europe. There are presently no full TLA systems in the UK.

Molecular biology in laboratory medicine Molecular biology has a vital role to play in the diagnosis and management of many disorders, and the number of applications in laboratory medicine is increasing. Rapid genetic techniques are superseding many existing conventional phenotypic tests, with marked improvements to the service provided to patients, often with savings in staff time and associated costs. In haematology, genetic analysis provides important information that may not be obtainable by other methods, for example accurate and timely carrier and prenatal diagnosis in haemophilia or thalassaemia, screening for genetic risk factors in thrombophilia, and molecular monitoring in leukaemia. There are also many uses for molecular biology techniques in other laboratory medicine disciplines, such as in the diagnosis and treatment of infection in microbiology/virology. Applications include testing for hepatitis and other viruses, monitoring the impact of new vaccines, such as meningitis vaccine, and evaluating the response to HIV therapy.

Following a process of national competitive bidding against the Department of Health's Pathology Modernization Fund for the year 2000–2001, CMHT was awarded £965 000 to develop an integrated molecular diagnostics facility in laboratory medicine. The bid represented a partnership between the Directorate of Laboratory Medicine at CMHT, the Manchester PHLS, the Royal Manchester Children's Hospital and the Infectious Diseases Unit at North Manchester General Hospital.

The new, purpose-designed molecular biology laboratory, the 'Manchester Molecular Diagnostics Centre', became operational in July 2001. The laboratory, which includes state-of-the-art equipment such as robotic sample handling devices and an automated capillary DNA sequencer, will facilitate the development of a cross-laboratory medicine molecular diagnostics service provision for Manchester and the surrounding areas. Equally, it will provide a high-class environment for clinical molecular biology research and development.

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UK NEQAS for leucocyte immunophenotyping: past, present and future

Introduction External quality assessment (EQA) is now accepted as an integral component of the clinical laboratory's activity. Indeed, such activities are likely to assume even greater importance following the implementation of the Clinical Pathology Accreditation's (CPA) revised standards which specifically highlight the need for appropriate participation and state that EQA performance should be reviewed and communicated to staff and the decisions taken recorded, monitored and acted upon. To ensure adequate EQA provision, UK NEQAS for Leucocyte Immunophenotyping, like all EQA providers, is subject to 2-yearly CPA (EQA) accreditation inspection. In addition, the scheme has continued to broaden its scope to meet clinical demands, including the development of a pilot scheme for low-level leucocyte counting and an exploratory scheme for molecular haematolo-oncology diagnosis. A specialist advisory group (SAG) has also been established to provide appropriate clinical and scientific support (for overview, see Reilly & Barnett, 2001).

The UK NEQAS for Leucocyte Immunophenotyping has become the largest international scheme of its kind (providing EQA to more than 650 laboratories, in more than 20 countries) incorporating assessment programmes for leukaemia immunophenotyping (immunocytochemistry and immunoflorescence) as well as for CD34+ peripheral blood stem cell, CD4+ T cell and low-level leucocyte counting. A crucial factor in the success of the scheme has been the development of a unique stabilizing process that ensures the retention of leucocyte light scatter and immunological staining characteristics for up to 300 days. Previously, EQA programmes have used either fresh whole blood or frozen cells. The instability of such analytes means that samples need to be shipped by express courier, making transportation costs prohibitive. More importantly, wide CVs are seen with the use of non-fixed material, a fact that may potentially conceal the identification of crucial elements for satisfactory laboratory performance.

Leukaemia immunophenotyping This scheme has consistently highlighted analytical problems, including the inappropriate use of fluorochromes, fixatives and antibody titre, and the identification of effective gating strategies, all of which have contributed directly to the improvement in interlaboratory variation seen since the commencement of the scheme. Nevertheless, EQA schemes for immunophenotyping will need to evolve as recent advances in reagents and techniques become routine practice. For example, the past five years has seen the development of novel antibodies (e.g. anti-CD79a/b and anti-CD117), the widespread use of triple immunostaining, the commercial availability of permeabilizing reagents to enable the flow cytometric detection of intracellular antigens, the increasing clinical use of antigen quantification (Barnett *et al.*, 1998a), as well as gating strategies incorporating CD45 to allow the analysis of selected cell populations. These developments have resulted in the need for a revision of guidelines for acute and chronic leukaemia diagnosis (BCSH, in preparation).

CD4+ T cell enumeration The absolute numbers of CD4 lymphocytes and their percentage values within the total lymphocyte populations are the two most frequently requested flow cytometric assays which retain their clinical significance in HIV infections in four areas: (i) to assess the degree of immune deterioration and speed of progression towards AIDS; (ii) to group HIV seropositive naive patients into cohorts according to their baseline CD4 counts prior to initiating therapy; (iii) to properly choose the timing for prophylaxis of opportunistic infections; and (iv) to monitor the efficacy of antiviral therapy, in terms of CD4 recovery. UK NEQAS has shown, by using stabilized whole blood and strict adherence to gating strategies, that individual laboratory results for a given issue can be tightly distributed about the mean, as defined by a low SD value. In general, absolute T cell counts are calculated from the absolute lymphocyte count and the percentage T cell subset value; a method termed the 'dual platform approach'. However, UK NEQAS has shown that 'single platform' techniques produce consistently lower CVs and this approach is now the recommended method (Barnett *et al.*, 1999a). UK NEQAS has also developed 'Transfix', a fixative that can be added to fresh blood in order to secure longer travelling time to distant laboratories while maintaining optimal sample integrity. This will have important benefits for developing countries as it will allow optimal transport of specimens and, when used with inexpensive primary CD4 gating strategies, will aid economical patient management and support the current inexpensive antiretroviral therapy and vaccination programmes.

CD34+ stem cell enumeration Previous EQA programmes have reported interlaboratory CVs as high as 284%, suggesting the need for greater standardization. However,

with the use of a fixed preparation, UK NEQAS has demonstrated that interlaboratory CVs can, on an international scale, be reduced to less than 25% without using specified gating criteria (Barnett *et al.*, 1998b). However, our findings indicate that further improvements are possible if standardized protocols are adopted and highlight the urgent need for internationally agreed consensus protocols. These problems have been partially addressed in the UK by the publication of BCSH guidelines and their impact on UK laboratory performance is awaited with interest (Barnett *et al.*, 1999b).

Low-level leucocyte counting The routine practice of issuing blood components containing low levels of leucocytes was implemented by the UK National Blood Authority in 1999; mainly due to concerns about the potential risk of transmission of variant Creutzfeldt-Jacob disease (vCJD), but also to reduce or prevent adverse transfusion reactions, transfusion related sepsis and HLA alloimmunization. The UK specification for leucocyte depletion was set at $< 5 \times 10^6$ leucocytes per unit of whole blood or platelets as a risk reduction strategy. As a result of these developments, UK NEQAS for Immunophenotyping, in collaboration with the UK transfusion services, initiated an EQA programme for low-level leucocyte counting in 1998. Initial results highlighted the variability in low-level counting and emphasized the need for consensus protocols, including gating strategies and nuclear staining techniques (Barnett *et al.*, 2001). UK NEQAS has addressed these issues and produced not only a standardized gating strategy but also the development of a daily run control.

Future The next decade is likely to witness further significant changes to the Scheme. EQA of leukaemia immunophenotyping, for example, should evaluate all aspects of the diagnostic process, including data interpretation. To date, however, most EQA schemes have focused on assessing technical performance and, with the exception of the Dutch programme, have not yet addressed this issue. This will have to be addressed by the SAG as individual diagnostic performance will form an increasingly important aspect of clinical governance. Planned UK NEQAS projects include the development of a 'daily run' control standard for low-level leucocyte enumeration and a short-term fixative for use with clinical samples. Specific projects are underway to standardize clinical flow cytometry on an international basis. This project has been supported by the European Commission and will enable UK NEQAS to collaborate with partners from seven European countries to develop stable reference preparations for use in flow cytometry. Once developed, the reference materials will be deposited at national facilities and will be available for validating new assays and techniques. Ultimately, an infrastructure will be established to enable the development of a European wide network of EQA schemes.

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A role for induced heteroduplex generator analysis in postgenome SNP genotyping and haplotyping

DNA-based methods for detecting polymorphic sites in DNA have evolved rapidly, both in the pregenome and postgenome eras. The discovery of restriction endonucleases and DNA ligases was soon followed by their application to the creation of recombinant plasmids, phage and cosmids. Amplification of these recombinant vectors *in vivo*, in suitable hosts, was the early means whereby sufficient material could be obtained to permit DNA sequencing. For a significant period of time, the major tools for examining polymorphism were DNA sequencing and restriction fragment length polymorphism analysis, the latter by probing Southern blots with nick-translated or random-primed cDNA or cloned genomic DNA. The polymerase chain reaction (PCR) revolution in molecular biology was the platform technology responsible for the current plethora of methods for detecting single nucleotide polymorphisms

(SNPs). These can, however, be broadly categorized according to four main technical principles: direct sequencing; probe–target-based hybridization (including micro-arrays); allele-specific PCR (SSP, ARMS); and DNA conformational analyses (SSCP, heteroduplex analysis).

The human genome project has now provided the core data required for examining the whole genome for population-specific and, more importantly, disease-specific variation in humans. Postgenome methods for SNP analysis have, in many cases, been influenced by the requirement for high throughput analysis, with a concomitant need for complex and expensive instrumentation platforms and analytical software. However, neither direct sequencing, nor probe-target-based hybridization, nor allele-specific PCR analyses are readily adaptable to the analysis of *haplotypic* variation: i.e. they cannot define how two or more closely linked SNPs are arranged within heterozygous individuals. Many single-gene studies reveal not only high levels of individual SNPs, but also a highly complex haplotypic variation.

We have developed a PCR-based DNA conformational analysis method (induced heteroduplex generator analysis, IHG) that permits the analysis of haplotypes, gives unequivocal allele or haplotype calls, is rapid, simple and cheap, and can be used on a range of equipment/readout platforms, but which has been optimized for simplicity and cost-effectiveness to use on triple-wide polyacrylamide minigels (throughput 120 samples per run). Further key advantages of IHG technology are: a single IHG reagent can identify multiple SNPs within a target amplicon; previously undescribed SNPs can be identified within an amplicon; a complete series of controls, representing all known SNPs, are included with each IHG such that the end user can validate the tests and be confident of calling genotypes.

To discriminate between two alleles, an IHG is synthesized, which is an exact copy of one of the alleles except that a poly adenosine insert is included adjacent to the base representing the polymorphic residue. In the test, gene fragments containing the SNP are amplified using the PCR. The IHG is amplified separately. Amplicons from the gene fragments under test and the IHG are mixed, heated, and cooled to allow heteroduplexes (mismatched hybrids) to form. These heteroduplexes differ in molecular conformation and permit the identification by electrophoresis of alleles in any combination. Where SNPs are clustered, the IHG is designed with more than one insertion site and thus a single IHG can identify a complex arrangement of SNPs.

The following list represents some the many applications of IHG technology to date.

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Type 1 von Willebrand's disease: possible genetic causes

von Willebrand's disease (VWD) is the commonest inherited bleeding disorder in humans, reported to occur in 1% of some populations. It is caused by qualitative and/or quantitative deficiency of plasma von Willebrand Factor (VWF), a multimeric, multifunctional plasma glycoprotein responsible for effective primary haemostasis (through subendothelial-platelet and platelet–platelet interactions) and for protecting clotting factor VIII in from premature proteolysis in the circulation. VWF is synthesized in vascular endothelial cells and megakaryocytes. Three types of VWD are recognized; type 1 is the commonest (70%) and is generally clinically mild resulting from reduced levels of qualitatively normal VWF; type 2 consists of patients with generally normal levels of variants of VWD affecting binding to platelets or to FVIII; type 3 is a rare, severe condition characterized by very low or undetectable plasma VWF levels. Types 1 and 2 are generally inherited in an autosomal dominant fashion (except type 2 N, which exhibits reduced VWF-FVIII and is inherited in a recessive form). Type 3 is also autosomal recessively inherited with patients being either homozygous or compound heterozygous for mutant alleles.

The VWF gene found on chromosome 12 is 181 kb in length and has 52 exons. Its analysis is complex and has only recently been achieved in a significant number of patients. 80% of type 3 patients have mutations which result in failure to produce VWF (VWF gene deletions, STOP codons, frameshifts and splice site changes). Type 2 patients have missense mutations in particular regions of the VWF gene encoding functional domains.

Very few mutations within the VWF gene have been identified to date in type 1 patients particularly where dominant inheritance is clear. This is despite the increasing ability by several laboratories to analyse the complete VWF gene (exons, introns, exon-intron boundaries, 5' and 3' flanking regions). The possibility of a dominant negative mechanism has been explored and only confirmed in one case where Cytosine 1149 is replaced by Arginine in the D3 domain of VWF

(Eikenboom *et al.*, 1996). A second reported mutation (C1130F) also probably operates by a similar mechanism. Our own studies in Sheffield have identified a mutation in a patient with type 1 VWD (R273W in the propeptide region), which, when expressed in COS-7 cells results in reduced cellular secretion when compared to wild type VWF resulting from increased binding to intracellular chaperones within the ER system (Allen *et al.*, 2001).

It is, however, becoming increasingly clear that the failure to detect VWF gene mutations in the VWF gene in type 1 patients may not be related to technical problems but to the fact that mutations may not be present within the VWF gene in these cases. Thus in a linkage study of 10 previously reported type 1 VWD families in Northern Italy, only three showed evidence of linkage between VWD and the VWF gene, two showed possible linkage and five showed no linkage.

VWF gene haplotypes may, through haplotype/haplotype interactions, influence VWF plasma levels, and, significantly, VWF gene promoter polymorphism haplotypes have been reported to affect VWF plasma levels particularly in adults older than 40 years (Keightley *et al.*, 1999). Non-expressed VWF alleles, the hallmark of type 3 VWD are not a common cause of type 1 VWD (Coughlan *et al.*, 1999).

ABO blood group status is clearly linked to VWF plasma levels both in normal individuals and in patients with VWD. Type O individuals have an average of 25% less plasma VWD than non-O individuals (Gill *et al.*, 1987). It is increasingly believed that changes in genes closely related to VWF production and metabolism (e.g. glycosyltransferases) may have affect on the secretion/survival of VWF in plasma and be associated with type 1 VWD. The example of a mouse model of VWD where an inappropriate expression of a glycosyltransferase gene in the vascular endothelial cells appears to result in plasma VWF with an abnormal glycosylation pattern and reduced survival (Mohlke *et al.*, 1999) is unlikely to be mirrored in humans. However the concept of different glycosylation patterns linked to VWF gene haplotypes is a distinct possibility.

VWD was first described in Europe. Under the European Commission FW5 programme, we have obtained funding for a collaborative project to study molecular and clinical markers for the diagnosis and management of type 1 VWD. This involves 12 centres which hope to recruit up to 200 families with type 1 VWD. Detailed phenotypic and genotypic analysis will be performed in order to provide precise data on which to base VWD type 1 diagnosis. It is also expected that the study will reveal a significant number of families where VWF gene-VWD linkage will not be present, so providing a resource for the study of other factors implicated in this disease.

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Can haematology laboratories predict thrombosis?

Introduction The value of haematological tests in predicting thrombotic events is best estimated by collaborative meta-analyses of prospective studies. This minimizes bias, estimates the degree of uncertainty of the prediction, indicates what further research is required to reduce uncertainty, and thus helps to estimate clinical value of the tests. This review concentrates on blood levels of variables, this reviewer taking the old-fashioned view that such phenotypes potentially indicate function. Reviews of thrombotic genotypes (which may or may not indicate function) have been published recently (Reiner *et al.*, 2001).

Blood rheology Haematological tests of blood flow properties include haematocrit, viscosity (plasma or whole-blood) and measures of red cell aggregation (traditionally the

erythrocyte sedimentation rate, ESR). A recent meta-analysis (Danesh *et al.*, 2000) showed that each of these variables is a predictor of arterial thrombosis (coronary heart disease). Haematocrit and viscosity may also be predictors of stroke Lowe *et al.*, 1997), as well as dementia (Elwood *et al.*, 2001a; Lowe, 2001a). Plasma viscosity and ESR are determined not only by fibrinogen and other high molecular weight reactant proteins, but also by lipoproteins (Lowe, 2001a, b). Statins reduce viscosity by reducing lipoproteins (Lowe, 2001b); while viscosity can also be lowered by fibrinogen reduction with fibrates or with ancrod: the latter may improve the outcome in thrombotic stroke, and reduces the risk of venous thromboembolism in immobilized elderly medical or surgical patients (Lowe, 1997a).

Platelets Recent studies suggest that neither platelet count nor platelet aggregation tests predict arterial thrombosis (coronary heart disease or stroke) (Meade *et al.*, 1997; Elwood *et al.*, 2001b; Lowe, 2001b) or venous thrombosis (Lowe, 1997b). While the results of antiplatelet trials clearly indicate a role of platelets in both arterial and venous thrombosis, it may be that extrinsic factors are more important (e.g. haematocrit, fibrinogen, von Willebrand factor (Danesh *et al.*, 1998, 2000; Whincup *et al.*, 2001).

Coagulation Recent meta-analyses have shown that fibrinogen (Danesh *et al.*, 1998), von Willebrand factor – factor VIII complex (Whincup *et al.*, 2001), and fibrin D-dimer (Danesh *et al.*, 2001) (a marker of cross-linked fibrin turnover) are predictors of arterial thrombosis (coronary heart disease). There is increasing evidence that these variables are also predictors of stroke, and of venous thrombosis (Lowe, 1997b; Lowe *et al.*, 1999). von Willebrand factor can be modified by antibodies, factor VIII increases by beta-adrenergic blockers, and D-dimer by anticoagulants.

Coagulation inhibitors There is now little doubt that classical hereditary thrombophilias (as a result of deficient coagulation inhibition) are associated with increased risk of venous thrombosis (Reiner *et al.*, 2001). However, the value of screening remains to be established. Furthermore, there is little evidence that they predict arterial thrombosis (Reiner *et al.*, 2000).

Fibrinolysis A recent meta-analysis showed that tissue plasminogen activation (t-PA) antigen levels (but not PAI-1 levels) are predictors of arterial thrombosis (coronary heart disease) (Lowe *et al.*, 2001), possibly because t-PA is up-regulated in arterial plaques, and may play a role in plaque rupture.

Country and lifestyle Recent evidence suggests that some of these tests correlate with both international variation in CHD risk, and also with lifestyle (e.g. physical activity).

Conclusion There is increasing evidence that haematology laboratories can predict thrombosis. The debate is now: which, who and why?

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Promoting clinical science in clinical practice

Clinical science enters the 21st century with a mixed collection of baggage. On the one hand, the high level of public interest in healthcare and significant, well-publicized advances such as the Human Genome Project create a climate that should attract good scientists into clinical science. On the other hand, an increasing distrust of both scientists and the medical profession, and the perception of a 'third world health service' is in danger of diminishing public confidence and turning potential recruits away.

The foreword to *Making the Change – A Strategy for the Professions in Healthcare Science*, the government's human resource strategy document published in February 2001, states 'The NHS employs some of the UK's most talented scientists, engineers and technologists and provides them with the opportunity to contribute creatively to a scientifically based health system.' It sets out proposals for the professions working in healthcare science, and indicates how scientific staff can be supported and developed to benefit patients and to promote innovation in healthcare. However, there are responsibilities attached to the benefits. *Making the Change* also requires scientists 'to be flexible in the way they work, breaking down professional barriers to develop further their skills and talents, always against a background of validated competence'. The challenge will be to deliver this flexibility while preserving the high quality of specialist expertise currently available to the NHS, and ensuring that greater flexibility does not mean 'dumbing down'.

There is now government recognition that clinical science provides the evidence base that underpins evidence-based medicine, and that without effective laboratory scientific services it will be impossible to deliver the National Service Frameworks and the modernized NHS envisaged in the NHS Plan. This recognition must now be channelled into effective action to address the staffing crisis in the service, strengthen and modernize education and training, ensure attractive career pathways so that high quality staff can be recruited and retained, and develop the research capacity of the NHS.

Proper workforce planning is essential to solve the staffing problems of the scientific service, and requires a clear understanding of what roles need to be performed to support clinical diagnosis, monitoring and research. The specific needs of small professions will need to be supported by the new local Workforce Development Corporations, and a much more coherent approach to workforce planning is required.

Increasingly, the scientific workforce, just like the medical profession, will need to demonstrate that it has been trained fit for the tasks undertaken. This will require detailed definition of competences, and the National Occupational Standards Project in Healthcare Science has been developed to define the specific competences required for the various roles within the service. This will provide the basis for transfer between professions while (one hopes) protecting the specialist skills required to interface clinical science with clinical medicine.

The government is also determined to modernize and develop professional regulation, to provide proper protection for patients and the public. Most of the scientific workforce is currently regulated by the Council for the Professions Supplementary to Medicine, which will be replaced in 2002 by the Health Professions Council (HPC). The HPC will regulate some 120 000 healthcare professionals, including 40 000 who are designated as the scientific professionals. It will have much wider powers to deal with practitioners who are felt to endanger the public, including the ability to withdraw registration from practitioners who fail to maintain competence or who are judged incapable of practice on health grounds. Revalidation for scientific professionals is thus developing along similar lines to the General Medical Council's proposals for the medical profession.

In parallel with these proposals, the *Agenda for Change* proposals to modernize the NHS pay system and the proposals in *Making the Change* to develop the career structure have the potential to improve recruitment and retention, and provide staff with proper rewards and appropriate opportunities for advancement. All the building blocks are now in place, but the changes will need to be properly managed and adequately resourced if real progress is to be made.

There is much to be done over the next five years, but there are now clear opportunities to revitalize the NHS scientific service and to create a structure that continues to lead the way in research, diagnosis and treatment.