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CONCOMITANT MYELODYSPLASTIC SYNDROME AND CHRONIC MYELOID LEUKAEMIA: TREATMENT OUTCOMES WITH IMATINIB MESYLATE

A 74-year-old man with slowly progressive leucopenia, macrocytic anaemia and thrombocytopenia over a 10-year period presented at our institution in September 2001 with a haemoglobin 9.8 g/dl (transfused), leucocyte count of $1.4 \times 10^9/l$ (18% neutrophils, 82% lymphocytes) and a platelet count of $42 \times 10^9/l$ without evidence of splenomegaly by examination. A bone marrow biopsy was hypercellular (80%) and demonstrated trilineage dysplasia with no increase in myeloblasts. Conventional G-banded chromosome analysis revealed a karyotype of 46,XY,del (20)(q13.1) in all 20 metaphases examined. The patient was given a diagnosis of refractory cytopenias with multilineage dysplasia (RCMD; International Prognostic Scoring System stage of intermediate-1; Greenberg *et al*, 1997), began a course of palliative erythropoietin for his anaemia and was followed. In July 2002, he developed an increased leucocyte count with marked circulating immature myeloid cells (leucocyte count $9.1 \times 10^9/l$; 36% myelocytes, 12% metamyelocytes, 6% undifferentiated blasts, 9% progranulocytes, 16% segmented neutrophils, 26% lymphocytes and 1% monocytes), progressive splenomegaly, lymphadenopathy and new cutaneous lesions (erythematous plaques). Biopsy of the lymph nodes and skin lesions revealed infiltrating myeloid progenitors consistent with extramedullary haematopoiesis. A repeat bone marrow biopsy was markedly hypercellular (100%) with an increase in blasts (10%), suggesting progression of his myelodysplastic syndrome (MDS) to refractory anaemia with excess blasts. In comparing the two recent bone marrows, the morphological features were similar. Both were hypercellular on account of panhyperplasia with trilineage dysplasia. The most prominent changes were the development of neutrophilia and an increase in bone marrow blasts. Although frequent, small, hypolobated megakaryocytes were present in the second specimen, as often seen in chronic myeloid leukaemia (CML), they were also seen in the first specimen. Cytogenetic analysis again showed a deletion of chromosome 20q in each metaphase, but also the interval development of a subclone of cells (three out of 20 metaphases) with the karyotype 46,XY,del (20)(q13.1),t(9;22)(q34;q11.2). Similarly, fluorescent *in situ* hybridization (FISH) studies on peripheral blood showed 38.4% of 500 nuclei with *BCR/ABL* fusion [double fusion FISH (D-FISH) strategy, normal <1%]. Owing to the marked activity of imatinib mesylate (Gleevec; Novartis Pharmaceuticals, Hanover, NJ, USA) therapy in patients with CML and t(9;22)(q34;q11.2) (Druker *et al*, 2001), the patient was begun on imatinib mesylate therapy at 400 mg/d.

The patient remained on imatinib for 6 months. During this time, the percentage of cells in his blood with *BCR/ABL* fusion by FISH fell from 38.4% to a minimum of 9.8%. However, the patient had no improvement in his anaemia or thrombocytopenia. Indeed, additional attempts to escalate the dose of imatinib further, to gain a greater response, were hindered by dose-limiting thrombocytopenia. Secondary to the persistent thrombocytopenia, attempts were made to discontinue the imatinib mesylate, which resulted in marked leucocytosis (felt to be secondary to his CML phenotype) ($130 \times 10^9/l$) that resolved with reinstitution of therapy (see Fig 1), demonstrating that he did indeed manifest a CML phenotype concurrent with his MDS. In January 2003, the patient developed pneumonia and died. Post-mortem examination demonstrated an infiltration of myeloid progenitors in all major organs.

The development of a Philadelphia chromosome associated with the typical t(9;22)(q34;q11.2) in patients with MDS is a very rare, but previously reported, event and is possibly a consequence of the acquired genetic instability associated with MDS (Verhoef *et al*, 1992). Goldberg *et al* (2003) recently reported the progression of an MDS subclone in a patient with CML treated with imatinib mesylate. Suppression of the Ph⁺ clone by imatinib mesylate led to a complete haematological response with respect to CML and normalization of marrow FISH-based studies for the *BCR-ABL* translocation, yet karyotyping revealed evolution of the MDS subclone in the marrow. In order to assess the frequency of patients with a t(9;22) subclone, a retrospective review of the Mayo Cytogenetics Laboratory database was performed (included both Mayo Clinic patients and

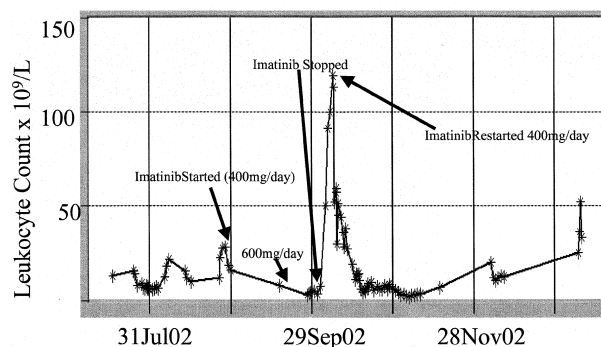


Fig 1. Leukocyte response to imatinib mesylate therapy in a patient with MDS with a secondary t(9;22)(q34;q11.2) subclone.

specimens referred to Mayo Medical Laboratories). A t(9;22) was identified by conventional karyotype analysis in 659 different patients over a 3-year period. Of the 659 patients (including the patient described above), only one other patient had a t(9;22) as a subclonal event, indicating a frequency of 0.3% (two out of 659) for the acquisition of this secondary anomaly. This second patient's primary karyotypic anomaly was t(3;21)(q26.2;q22), a translocation previously associated with genotoxic chemotherapy, which may represent a medication-related event (Zent *et al*, 1997).

The findings in the present case and the patient described by Goldberg *et al* (2003) suggest several conclusions when multiple clonal myeloid processes exist in the marrow. First, a Ph⁺ subclone appears to result in phenotypic features typical of CML, including organomegaly, leucocytosis and extramedullary haematopoiesis, even within a background of MDS. Secondly, imatinib mesylate is able to suppress a t(9;22)-driven clonal process even when this process is secondary in origin, although this suppression may be incomplete. Finally, suppression of a t(9;22) clonal process in a patient with MDS does not necessarily alleviate the manifestations of MDS, such as other cytopenias, and the MDS may progress clinically.

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Keywords: myelodysplastic syndrome, chronic myeloid leukaemia, imatinib mesylate, t(9;22) subclone.

SEVERE ACUTE RESPIRATORY SYNDROME AND LUPUS ANTICOAGULANTS IN CHILDREN

Severe acute respiratory syndrome (SARS) caused by the novel corona virus is a new emerging disease that has affected countries worldwide, with more than 8461 cases reported (WHO, 2003a; World Health Organization Multicentre Collaborative Network for Severe Acute Respiratory Syndrome (SARS) Diagnosis, 2003). Between 24 March and 31 March 2003, the United Christian Hospital admitted a total of 21 paediatric patients, age range from 11 months to 17 years, who fulfilled the WHO definition of SARS (WHO, 2003b). There were 10 boys and 11 girls. Eighteen patients were from the major outbreak in Amoy Gardens, a high-rise housing estate in Hong Kong.

In our group of 21 paediatric patients, we detected an unexpected, isolated, prolonged activated partial thromboplastin time (APTT) on admission in nearly half the cases. Eight patients (38.1%) showed a prolonged APTT of > 38.6 s, which is 1 standard deviation (SD) above the upper normal limit (reference range 24.1–35.7 s). The prothrombin time was normal in all patients. The fibrinogen level was within the normal range (2.0–4.0 g/l) in all 16 patients tested. D-Dimer was negative in 18 patients, positive (0.5–1.0 µg/ml) in two patients and not done in one patient. Further investigations were performed in five of the eight patients, and the results are given in Table I. Factor VIII levels ranged between 0.84 and 1.10 IU/ml (mean 0.96 IU/ml). Factor IX levels ranged between 0.60

Table I. Results of investigation of patients with a prolonged APTT.

Patient	Sex/ age (years)	APTT (s)	Lupus anticoagulant test* (ratio)†	Factor VIII (IU/ ml)	Factor IX (IU/ ml)
1	M/13	54.9	Negative (1.09)	0.94	0.74
2	M/15	51.9	Borderline (1.19)	1.10	0.62
3	M/15	44.9	Weakly positive (1.35)	0.84	0.60
4	F/16	42	Weakly positive (1.35)	0.93	0.72
5	M/13	39.2	Weakly positive (1.46)	0.97	0.71

*Lupus anticoagulant (LA) measured with the LA1 screening reagent and LA2 confirmation reagent (Dade Behring, Marburg, Germany).

†Ratio of LA1/LA2. Ratio between 1.1 and 1.3, results are borderline; ratio between 1.2 and 1.5, LA is weakly positive.

and 0.74 IU/ml (mean 0.68 IU/ml). All factor assays showed non-parallelism, indicating the presence of a non-specific inhibitor. A weak lupus anticoagulant was identified in three patients. The prolonged APTT, found in eight cases, had normalized by d 8 of admission. There were no thrombotic complications, bleeding events or deaths recorded in our group of patients.

So far, only one study has reported a prolonged APTT in SARS patients. (Lee *et al.*, 2003) Our investigations showed that prolonged APTT in paediatric SARS patients is not due to disseminated intravascular coagulation, but is rather caused by the presence of lupus anticoagulants. Lupus anticoagulants are members of a heterogeneous group of antibodies directed against phospholipid protein complexes and can arise after events triggering the immune system. These antiphospholipid antibodies have been found to be associated with many infections, although the pathogenic role for these antibodies has not usually been obvious (Asherson & Cervera, 2003). We have not repeated the lupus anticoagulant testing to determine whether the phenomenon is transient or permanent. As SARS is a new disease, the cause and pathogenic role of lupus anticoagulants or other antiphospholipid antibodies remain to be studied.

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Keywords: severe acute respiratory syndrome (SARS), lupus anticoagulants, prolonged activated partial thromboplastin time.

MALIGNANCIES IN SICKLE CELL DISEASE PATIENTS TREATED WITH HYDROXYUREA

We read with great interest the recent review on hydroxyurea (HU) in sickle cell disease (SCD) (Halsey & Roberts, 2003). As a minor point, we would like to correct the fact that the patient who developed a lymphoproliferative disease did not belong to the Belgian study, but was reported by the French group (De Montalembert & Doures, 2001).

However, the first case of a SCD patient who developed malignancy has just been reported to the Belgian Registry of Sickle Cell Disease patients treated with hydroxyurea. The patient is a 21-year-old woman who has been treated with HU for the last 8 years for severe clinical disease (recurrent vaso-occlusive crises, osteonecrosis, acute chest syndrome). Treatment with HU was discontinued 2 years ago because the patient was pregnant and was restarted in July 2002 after she had given birth to a healthy daughter. In March 2003, the patient complained of diffuse bone pain; a blood examination showed leucopenia and thrombocytopenia, which persisted after discontinuation of HU. Bone marrow examination confirmed the diagnosis of acute myeloblastic leukaemia M3v.

This is the first case of HU-associated malignancy in the SCD Belgian Registry. To date, 141 patients have been included, and 598 'patient-years' have been evaluated with a median follow-up of 4.5 years.

Several reports suggest that SCD patients may be at either lower or higher risk for malignancy (Jackan, 1972; Stricker *et al.*, 1986; Paydas, 2002). The contribution of HU in the emergence of neoplasia in SCD patients is

currently unknown. However, our case, together with those reported by Raunch *et al.* (1999) and Wilson (2000), emphasizes the need for close monitoring, data collection and follow-up of SCD patients in order to determine the risks and benefits of any treatment in this particular population.

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Keywords: sickle cell disease, hydroxyurea, malignancy.

LITHIUM EFFECTS ON NEUTROPHIL MOTILITY IN SHWACHMAN–DIAMOND SYNDROME: EVALUATION BY COMPUTER-ASSISTED IMAGE ANALYSIS

In a recent review of Shwachman–Diamond syndrome (Dror, 2002), the author cited our previous papers concerning the ability of lithium to restore neutrophil chemotaxis, evaluated by conventional methods (Azzarà *et al.*, 1988, 1991). After those reports, we developed a very sensitive, computer-assisted image processing technique, capable of investigating the neutrophil migration kinetics when applied to the micropore filter method in the Boyden chamber (Azzarà *et al.*, 1992, 1995). According to this technique, the random propagation of normal neutrophils is defined by a Gaussian curve; in the presence of a chemotactic stimulation, the migration is characterized by a very typical pattern, with a maximum 'peak' located beyond the initial plane (Fig 1).

Therefore, we re-evaluated all filters of the tests carried out in the aforementioned papers. In addition, several further tests during the follow-up (between 1992 and 1998) were performed in the same patient, who underwent discontinuous lithium therapy. We would like to report the information furnished by these additional observations.

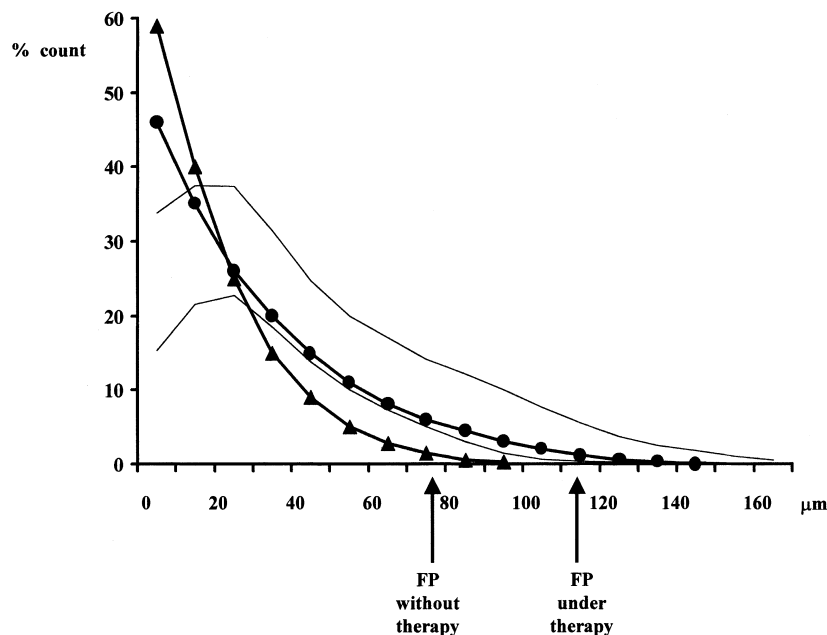
The *in vitro* study, carried out in 1988 (Azzarà *et al.*, 1988), had shown that lithium was able to enhance

neutrophil chemotaxis from 87 μm to 103 μm , without interference with random motility. The *in vivo* study, carried out in 1991 (Azzarà *et al.*, 1991), confirmed that lithium did not interfere with random motility, which was normal in basal conditions, either after 10 d therapy or after 30 d therapy. On the contrary, it was able to enhance neutrophil chemotaxis, which was strongly inhibited in basal conditions (77 μm), up to 102 μm after 10 d therapy and up to 111 μm after 30 d therapy [normal value $109 \pm 7 \mu\text{m}$ (2 SD)]. Both studies had been performed by the conventional 'leading front' technique, which can only measure the distance travelled by the cells; therefore, no information was available about the kinetics of migration.

When the same filters were examined later by the image analysis workstation, we confirmed the data regarding random migration, which also exhibited a consistent normal Gaussian pattern. However, interesting data were obtained regarding chemotaxis. In fact, although the measures of the distance travelled by the cells were very similar to those obtained using the conventional method (76, 104 and 115 μm respectively), neither a Gaussian pattern (which would have indicated an unspecific enhancement of chem-

Lithium effects on neutrophil chemotaxis in Shwachman–Diamond Syndrome

Fig 1. Plots of the curves that interpolate the average cell counts throughout micropore filters during chemotaxis. Continuous curves, kinetics of normal donors' neutrophil migration (range: mean $\pm$ 2 SD). Continuous curve with triangles, kinetics of the patient's neutrophil migration without therapy. Continuous curve with circles, kinetics of the patient's neutrophil migration under therapy. Final plane (FP), the distance travelled by neutrophils, calculated by the algorithm after: (1) decimal logarithm transformation of counts (ordinate); (2) square transformation of depth (abscissa); (3) calculation of the regression line; (4) square root of the interception values on the abscissa, obtained when the logarithm value decreases by two units. FP without therapy, $79 \pm 9 \mu\text{m}$ (mean of nine evaluations performed over a period of 4 years). FP under therapy, $115 \pm 8 \mu\text{m}$ (mean of six evaluations performed over a period of 3 years) ($P < 0.001$).



okinesis) nor a typical chemotactic pattern was obtained; this indicates that the improvement was probably associated with a neutrophil subpopulation, whereas the majority of cells remained inhibited on the initial plane. The patient then cyclically discontinued and restarted therapy (four periods and three periods respectively). During the off-therapy periods, neutrophil chemotaxis was always inhibited: $79 \pm 9 \mu\text{m}$ (mean of nine evaluations performed over a period of 4 years). During lithium therapy, it was always normal: $115 \pm 8 \mu\text{m}$ (mean of six evaluations performed over a period of 3 years) ($P < 0.001$). In addition, the kinetics of the neutrophils was always identical to that found in the basal condition. In particular, it can be seen that the typical peak of maximum cell density beyond the first plane was never detectable (Fig 1).

Our data confirm a clear activity of lithium on neutrophil motility in this syndrome. The drug did not affect chemokinesis, so it is not able to enhance random motility. Under chemotactic conditions, it probably acts by the recruitment of a subset of neutrophils unaffected by the cytoskeletal/microtubular abnormality, which is presumed to play a prominent role in the causation of defective chemotaxis (Rothbaum *et al.*, 1982). Of interest, most neutrophils remained insensitive. So it can be stated that, even if the function is ameliorated, the cytoskeletal defect represents a specific stable marker of the syndrome.

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Keywords: lithium, neutrophil motility, image analysis.

PROGNOSTIC FEATURES OF SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES

The very interesting paper by Parry-Jones *et al* (2003) on the prognostic features of splenic lymphoma with villous lymphocytes (SLVL) adds to our knowledge of this intriguing disease. However, in our opinion several points deserve further comments.

While 240 patients were identified, complete data were available for only 53% of them. This suggests a note of caution about the reliability of the prognostic evaluation because selection bias cannot be ruled out.

In addition, we were surprised to note that none of the cases showed an intrasinusoidal infiltrate in the bone marrow, which is highly characteristic and rather frequent in SLVL (Franco *et al.*, 1996; Costes *et al.*, 2002; Iannitto *et al.*, 2002).

Another point we would like to comment on is how the causes of death were reported. The 22 patients who died of progressive disease or infection certainly represent 17% of all the series, but it would have been more informative to underscore the fact that they made up 43% of all deaths. Unfortunately, the authors reported the occurrence of secondary cancer in their series, but did not specify the number or histotype of the cases involved.

The predictive value for survival of the covariates at diagnosis was reported, considering both lymphoma-related deaths and all other deaths (Table III in Parry-Jones *et al.*, 2003), but the causes of death were unavailable in as many as of 29% of cases (15 of 51). We believe that it cannot be assumed that none of these patients died of lymphoma-related causes. Therefore, the results of the Cox regression analysis indicating that, for lymphoma-related deaths, only haemoglobin level and lymphocyte count were independent prognostic indicators of survival, in our opinion should be taken cautiously. It could be misleading.

The last point is that of therapy. Parry-Jones *et al* (2003, Table II) reported the presenting features of treated and untreated patients. Patients who received chemotherapy as first-line treatment had a significantly higher lymphocyte count than those who were splenectomized. This means that they were at higher risk of lymphoma-related death according to the Cox analysis. Conversely, patients who received splenectomy as first-line therapy had a trend for a less-aggressive disease (younger age, lower total WBC count and lower degree of leucocytosis). It

would have been more informative if the data had been reported not as a median but as a percentage of patients with a high-risk covariate, using the cut-off described for the univariate analysis. Moreover, it is not clear how many patients that were not showing signs of active disease underwent splenectomy merely for diagnostic purposes.

We agree that patients at high risk at diagnosis because of lymphocytosis and anaemia do not seem to benefit from chemotherapy (mainly chlorambucil), but in our opinion Parry-Jones *et al* (2003) do not provide enough data to support the hypothesis that these patients may benefit from splenectomy.

We think that the issues of prognosis and the best therapeutic approach of SLVL deserve to be settled in a properly designed prospective study (Franco *et al*, 2003).

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Keywords: splenic marginal zone lymphoma, splenic lymphoma with villous lymphocytes, intrasinusoidal, splenectomy, bone marrow.

ERYTHROCYTOSIS AND THE CHUVASH VON HIPPEL-LINDAU MUTATION

Congenital and familial polycythaemias are rare conditions and the molecular basis of these disorders is largely unknown. The analysis of patients with familial erythrocytosis associated with low plasma erythropoietin (Epo) has detected mutations in the Epo receptor (EpoR) and thus has provided insights into the Epo signal transduction pathway.

The Chuvash form of polycythaemia describes a secondary familial and congenital polycythaemia characterized by normal or elevated levels of Epo. The molecular pathophysiology underlying the Chuvash polycythaemia has recently been elucidated and is ascribed to dysregulation of oxygen homeostasis caused by mutations in the von Hippel-Lindau (*VHL*) gene, a key player in the hypoxia-sensing pathway (Ang *et al*, 2002). Arg200Trp in *VHL* affects its association with a component of the hypoxia-inducible factor-1 (HIF-1) transcription complex and impedes the proteasomal degradation of this complex leading to increased expression of HIF-1 target genes, including *EPO* (Ang *et al*, 2002).

The patient, whose parents were Pakistani, was born in the UK in 1982. She required surgery at the age of 3 months with a haemoglobin (Hb) content of 12.6 g/dl then, and 18.3 g/dl at the age of 9 years. Mean cell volume (MCV), white blood cells and platelets were normal. Red cell mass was 52 ml/kg, plasma Epo level 18 mIU/ml (normal range 8–28), and both p50 and oxygen saturation were normal. The explanation for the polycythaemia was not obvious and she was treated by venesection. Over the next 9 years venesection was carried out with difficulty because of poor venous access. She was reviewed in 2000 and at that time she was well with a Hb of 15.0 mg/dl and MCV of 60. Her parents were not polycythaemic. Analysis of her clinical data suggested similarities to patients with the

Chuvash form of polycythaemia and sequencing of the *VHL* gene was performed. This showed that she was homozygous for the Arg200Trp mutation in the *VHL* gene. Both parents were heterozygous for this mutation.

Chuvash polycythaemia is not limited to Chuvashians. More recently, it has been shown that other mutations in *VHL* may lead to polycythaemia (Pastore *et al*, 2003) and that people of Asian and other ethnic origins, may also possess the same Arg200Trp *VHL* mutation described in Chuvash patients (Pastore *et al*, 2003; Percy *et al*, 2003). Analysis of the six previously described di-allelic single-nucleotide polymorphisms (Percy *et al*, 2003) revealed that the Arg200Trp mutation in our patient was associated with the same GCTACA haplotype as in the other Asian families (Percy *et al*, 2003), suggesting a founder mutation in the Asian population. Studies underway will establish whether the Arg200Trp mutation in the Chuvash and other ethnic groups is of a common origin.

Mutations in the *VHL* gene are better known in the context of the *VHL* disease, a dominantly inherited familial cancer syndrome. These patients have an increased incidence of renal cell carcinoma, central nervous system haemangioblastoma, pheochromocytoma and renal, pancreatic and endolymph cysts. The familial inheritance is related to a germline mutation in the *VHL* tumour suppressor gene followed by a second hit in *VHL* as a somatic mutation (Maher & Kaelin, 1997). Although more than 700 mutations have been characterized in *VHL*, no individual with two germline mutations of the type found in Chuvash polycythaemia (i.e. Arg200Trp) have been reported. Polycythaemia is not common but can occur as a paraneoplastic syndrome.

It is not clear whether patients with Chuvash polycythaemia or their heterozygous relatives are predisposed to neoplasms. There is some evidence to suggest that Chuvash polycythaemic patients may have increased morbidity and mortality from vascular events (Sergeyeva *et al*, 1997). Keeping the Hb to near normal levels is thus a priority and meticulous attention should be paid to maintaining good venous access from an early age. Eventually central venous lines, Hickman catheters, and portocaths may have to be considered, combined with some form of anticoagulation to reduce the risk of thrombosis in major veins. Arterio-venous shunts are another option. All such procedures are invasive and increase the risk of vascular complications. Finally, if all these methods fail bone marrow suppression by cytotoxic agents might be justified.

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Keywords: erythrocytosis, von Hippel-Lindau mutation, Chuvash polycythaemia.

ENDOTHELIAL CELLS EXPRESS NORMAL CELLULAR PRION PROTEIN

We read with interest the recent letter (Sivakumaran, 2003) in response to our article (Starke *et al*, 2002) in which the expression of normal prion protein (PrP^C) by endothelial cells was questioned. Sivakumaran's argument was that endothelial cells *in vitro* might not have the same phenotype *in vivo*. Specifically Sivakumaran believes that endothelial cells *in vivo* do not express PrP^C and that the expression previously reported could be the result of artefactual activation generated by *in vitro* manipulation. The reasoning behind this hypothesis was that paraffin sections of umbilical cord and adult blood vessels showed 'minimal or no expression of PrP^C' by immunocytochemistry using an antibody to prion protein (3F4).

We have demonstrated the expression of PrP^C in cultured human umbilical vein endothelial cells (HUVECs) and human microvascular endothelial cells (HMEC-1) by reverse transcription polymerase chain reaction (RT-PCR), flow cytometry and immunofluorescence microscopy using an antibody to prion protein (6H4). A time-resolved fluorimunoassay (DELFIATM, Perkin Elmer, Seer Green, Beaconsfield, UK) was used to demonstrate that endothelial cells released soluble PrP^C and human blood plasma also contained soluble PrP^C. By studying various groups of patients we demonstrated that plasma levels of PrP^C did not correlate with blood cell counts, leading to the conclusion

that the source of the plasma form of PrP^C may be the vascular endothelium.

Semi-quantitative RT-PCR was used to compare the expression of PrP^C mRNA in endothelial cells with the housekeeping gene iduronate-2-sulphatase (IDS). RT-PCR was performed using radioactively labelled primers and the amount of product quantified by densitometry. The mean expression of PrP^C relative to IDS was found to be 1.87 ± 0.75 for HUVECs and 2.97 ± 0.27 for HMEC-1s (Fig 1) ($n = 2$).

Simak *et al* (2002) demonstrated that cultured endothelial cells expressed PrP^C using several anti-prion antibodies. They also showed that endothelial cell microparticles expressed PrP^C. The expression of PrP^C by endothelial cells has been described in several other investigations (see Starke *et al*, 2002 and Simak *et al*, 2002 for reviews).

Sivakumaran used monoclonal antibody 3F4. This antibody is more restricted in the species of PrP^C that it can bind, compared with 6H4 (Barclay *et al*, 2002), and we found that it did not give as strong a signal with endothelial cells as 6H4 by flow cytometry (unpublished observations). Simak *et al* (2003) stated that the negative results obtained by Sivakumaran could be the result of a truncation of PrP^C in the tissue samples studied. We believe that a negative result should be confirmed with more than one antibody.

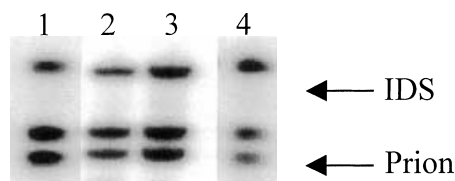


Fig 1. Semi-quantitative RT-PCR on endothelial cells. RT-PCR was performed on RNA extracted from HUVEC and HMEC-1 cells using radioactive (^{32}P) primers specific for iduronate-2-sulphatase (IDS) and PrP^C (prion). The products were then electrophoresed on a denaturing polyacrylamide gel, dried and exposed to hyperfilm. Lanes 1 and 2 are from HMEC-1s and lanes 3 and 4 are from HUVECs. Both bands labelled prion were found to be specific for prion mRNA ($n = 2$).

Erythrocytes were originally thought to be negative for PrP^C using antibody 3F4. It was later shown using antibody 6H4 that they do express low levels of PrP^C (Holada and Vostal, 2000). Immunocytochemistry is not a particularly sensitive technique and using neuronal tissue as a positive control for PrP^C expression may not reflect the sensitivity required for the study of tissues with lower prion levels. Regarding the artefactual expression as a result of *in vitro* activation, we have demonstrated that activation of endothelial cells with human thrombin and tumour necrosis factor- α did not increase the expression of PrP^C on endothelial cells and similar results were obtained by (Simak *et al.*, 2002).

In conclusion, we agree that there are limitations with regards to the study of endothelial cells *in vitro*, but that there is a great deal of data to indicate that vascular endothelial cells do express PrP^C. These cells remain a possible source of plasma prion protein and, as there is the possibility that PrP^{Sc} is transmitted via the haematogenic route, further research should be performed to determine the role of endothelial cells in patients with transmissible spongiform encephalopathies.

HEPARIN-INDUCED THROMBOCYTOPENIA: PATHOGENESIS AND MANAGEMENT

I read with much interest the recent review on the pathogenesis and management of heparin-induced thrombocytopenia (Warkentin, 2003).

Heparin-induced thrombocytopenia type II (HIT II) is a well-recognized complication of heparin therapy that can have serious effects. Several anticoagulation strategies have been developed for the management of HIT II patients who may require anticoagulation therapy.

Hirudin is natural peptide that was originally extracted from the salivary glands of leeches. Nowadays, it is made by recombinant DNA technology in yeasts. Recombinant hirudin (r-hirudin) has been used as a heparin substitute in a wide variety of clinical settings. It acts as specific direct inhibitor of thrombin. Despite its short half-life, its wider use has been limited because of the absence of a specific neutralizing agent.

In the section on lepirudin (Warkentin, 2003), the anticoagulation profile was monitored by the measurement

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Keywords: endothelium, prion protein, blood plasma, PrP^C.

of the activated partial thromboplastin time (aPTT). Several studies have shown aPTT assay to be of inadequate specificity to provide an accurate measurement of the anticoagulation effect of r-hirudin.

Pötzsch *et al.* (1997a) concluded, from a series of *in vitro* and *in vivo* experiments, that both the aPTT and the activated clotting time were not sufficiently sensitive to monitor the concentration of r-hirudin. However, there was a good linear relationship between r-hirudin concentration and the ecarin clotting time (ECT). Moser *et al.* (2001) arrived at the same conclusion from their experiments. The ECT is a parameter based on the conversion of prothrombin.

Ecarin is a prothrombin-activating enzyme that is derived from the venom of the snake *Echis carinaatus*. It activates the prothrombin to meizothrombin, which, in r-hirudin containing plasma, forms a stable 1:1 complex with r-hirudin. This results in a dose-dependent prolongation of the clotting time.

The ECT is a sensitive, easily performable assay for the precise measurement of the plasma r-hirudin level. Its precision is not affected by treatment with heparin, apro-tinin or oral anticoagulants (Pötzsch *et al*, 1997b).

There are numerous reports of the ECT assay providing rapid and accurate monitoring of the plasma r-hirudin concentration in patients with HIT II who underwent successful cardiopulmonary bypass operations using r-hirudin as the anticoagulant (Pötzsch *et al*, 1997a; Fabrizio, 2002; Saad *et al*, 2002).

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Keywords: heparin-induced thrombocytopenia, recombinant hirudin, anticoagulant therapy, ecarin clotting time.

HEPARIN-INDUCED THROMBOCYTOPENIA: PATHOGENESIS AND MANAGEMENT – RESPONSE TO RASHEED SAAD

Dr Saad correctly states that the activated partial thromboplastin time (aPTT) does not measure the anticoagulant effect of lepirudin as accurately as the ecarin clotting time (ECT). However, this advantage of ECT over aPTT is known to be relevant only in situations of very high lepirudin dosing, such as surgery requiring cardiopulmonary bypass (Warkentin & Greinacher, 2003). This is because the dose–response curve of the aPTT to increasing lepirudin concentrations flattens at high lepirudin concentrations (Greinacher, 2001). However, for most clinical situations, such as prophylaxis against or the treatment of thrombosis in acute heparin-induced thrombocytopenia (HIT), use of aPTT is believed to provide acceptable monitoring. Indeed, the pivotal clinical trials of lepirudin for the treatment of thrombosis complicating HIT used aPTT monitoring, with a target aPTT of $1.5\text{--}2.5 \times$ baseline for most aPTT reagents (Greinacher *et al*, 2000). Laboratories should assess their aPTT responsiveness of assay to increasing concentrations of plasma lepirudin: if the curve begins to flatten during the high therapeutic lepirudin concentrations (about 750–1000 ng/ml), then the physician should either aim at the low end of the aPTT target range (to avoid the potential for significant overdosing that might not be apparent if the aPTT lies within the high-therapeutic aPTT range) or utilize the ECT, if available. Whether the ECT might generally provide superior anticoagulation (i.e. greater therapeutic efficacy with less bleeding) than the aPTT in non-cardiac surgery situations

is unknown. This hypothesis would need testing in clinical trials comparing monitoring by aPTT versus ECT. However, even if the ECT was shown to be better, the aPTT might still remain the preferred monitoring method. Indeed, this is the current status for the monitoring of unfractionated heparin therapy in many clinical situations, although heparin levels can be measured more accurately by protamine neutralization assay or anti-factor Xa assay (Hirsh *et al*, 2001).

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