Thrombosis and acute lymphoblastic leukaemia

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Summary

Venous thrombosis is more frequent in patients treated for acute lymphoblastic leukaemia (ALL) than other malignancies and has distinctive causes, clinical features and remedies. The reported incidence varies from 1% to 36%, depending on the chemotherapy protocol and whether the reported cases are symptomatic or detected on screening radiography. The risk is thought to arise from increased thrombin generation at diagnosis combined with reduced thrombin inhibitory capacity due to depletion of circulating anti-thrombin (AT) by asparaginase. A number of patient and treatment variables have been reported to influence the risk of thrombosis including hereditary thrombophilia, early insertion of central venous catheters and exposure to a combination of steroids and asparaginase during induction. Erwinia asparaginase is associated with a lower risk of thrombosis compared with Escherichia coli asparaginase. The majority of symptomatic thromboses are related to central venous catheters and involve the upper venous system. Central nervous system thrombosis involving the cerebral venous sinuses is a unique feature of asparaginase-related thrombosis and is reported to occur in 1–3% of patients. Conclusive evidence to support the use of anti-coagulant treatment or AT concentrates for primary prevention is lacking, as is evidence for the efficacy of AT concentrates in the management of established thrombosis. Preventative strategies are hampered by conflicting data on factors that would enable identification of those at highest risk of thrombosis.

Keywords: thrombosis, acute lymphoblastic leukaemia, anti-coagulation.

Advances in the treatment of childhood acute lymphoblastic leukaemia (ALL) have resulted in cure rates of nearly 80% with modern intensive chemotherapy. However, intensive treatment imposes not only a considerable burden of morbidity (and for a small minority, mortality) but also limits delivery of drugs at optimal schedules and doses. The reduction of treatment-related morbidity, therefore, has become an increasing focus of clinical trials. Historically, this has been achieved through the use of risk stratification to identify high-risk groups for intensification of therapy. More recent trials are testing whether treatment can be reduced for precisely defined low-risk patients (among others, the UKALL 2003 trial. http://www.ctsu.ox.ac.uk/projects/leuk/ukall2003), a luxury afforded by the near certainty of cure for these groups with current treatment protocols. In addition, there is a growing understanding of the epidemiology and causes of toxicities gained from comprehensive adverse reporting systems within clinical trials. These have provided important insights into the complex interplay between host, disease and treatment-related factors in the pathogenesis of specific toxicities and are informing the development of prevention and treatment strategies. Thromboembolic events are among the more frequent and serious complications of ALL and its treatment, and represent a potentially reversible cause of morbidity and mortality.

This review covers the epidemiology, aetiology, investigation and management of thrombosis that occurs in association with ALL. The emphasis is unavoidably paediatric and relates to venous thrombosis as the reported data is almost exclusively from childhood ALL studies. The little adult data that is available indicates a similar pattern but a higher absolute risk of thrombosis (Elliott et al, 2004; Caruso et al, 2007).

The scale of the problem

Thrombosis is a well-recognised complication of ALL and its treatment in both adults and children. In children, ALL is the most frequent underlying cause of thrombosis in the absence of central venous catheters. Although less frequent than infection, bleeding or gut toxicity, thrombosis features among the more frequent serious adverse events reported in trials of ALL therapy. Whether thrombosis occurs more frequently in ALL than other cancers in adults is uncertain, as there are no studies comparing its incidence in different cancers. In children, non-central venous line (CVL)-related thrombosis is unusual in patients with solid tumours and is most often a presenting feature as a result of the compression of a proximate large vein by the tumour, whereas in ALL thrombosis is invariably treatment related and often affects the Central Nervous System (CNS).
Reported incidences for children receiving chemotherapy for ALL range from 1.7–36.7% (Gugliotta et al., 1992; Shapiro et al., 1993; Korte et al., 1994; Mitchell et al., 1994, 2003a; Sutor et al., 1999; Wermes et al., 1999a; Mauz-Korholz et al., 2000; Nowak-Gottl et al., 2001, 2003; Silverman et al., 2001). Table 1 summarises selected data on the epidemiology of thrombosis in patients with ALL. A recent meta-analysis of prospective studies in children with ALL found the global risk of symptomatic thrombosis to be 5.2% [95% confidence interval (CI) 4.2–6.4] (Caruso et al., 2006). These figures compare with a background annual incidence in children of just 1:100,000, illustrating the importance of ALL as a risk factor. The incidence of thrombosis in adult patients with ALL is less well studied. A retrospective review of 238 mostly adult ALL patients treated on the Gruppo Italiano per le Malattie Ematologiche dell’Adulti protocol ALL0288 reported an incidence of 4.2% (10/238) during induction (Gugliotta et al., 1992). A recent Californian study over a 6-year period found the incidence of venous thrombosis [exclusive of upper extremity deep vein thrombosis (DVT)] in 2482 patients (mean age 25.3 years) with ALL to be 3.7% with most events occurring within 3 months of diagnosis. This translated into an incidence rate of 2.5/100 patient years (Ku et al., 2006). Elliott et al. (2004) found a much higher incidence in a retrospective review of consecutively treated adults with ALL where 18.5% were reported to have had symptomatic, objectively confirmed thrombotic events during induction therapy. Caruso et al. (2007) published results of a meta-analysis of thrombotic complications in adults undergoing induction treatment for ALL. The meta-analysis included 13 prospective studies, which collectively reported 19 events in 323 patients. The incidence of thrombotic events during induction was 5.9% (95% CI: 3.5–9.2).

The wide variation in reported incidence is primarily due to differences in case ascertainment. Whereas retrospective surveys report clinically symptomatic thrombosis in <5% of cases, prospective studies with routine radiological screening report largely asymptomatic events in 11.5–36.7% of children with ALL (see Table I). The Prophylactic Antithrombin Replacement in Kids with ALL treated with Asparaginase study (PARKAA) reported a prevalence rate of 36.7% (95% CI: 24.4–48.8%) in 60 children screened whilst undergoing induction chemotherapy with a central venous catheter in situ. These were largely asymptomatic events diagnosed by screening with bilateral venography or magnetic resonance imaging (MRI), echocardiography and cranial MRI on completion of induction. In comparison, only three children (5%, 95% CI: 1.4%) had a symptomatic thrombosis (Mitchell et al., 2003a). Retrospective studies are also more prone to incomplete case ascertainment than prospective studies. A large retrospective multicentre survey of 1100 children treated on the Berlin Frankfurt Munster (BFM)-90 trial found 19 (1.7%) with thromboses (Sutor et al., 1999), whilst a smaller prospective study of similarly treated children showed a higher incidence of 14.3% (Korte et al., 1994). In the first 1242 patients entered into the current UK childhood ALL trial, UKALL 2003 (http://www.ctsu.ox.ac.uk/projects/leuk/ukall2003), the incidence of symptomatic thrombosis was 2.8% (CNS <0.6%) (trial database, A. J. Vora, unpublished data).

Events occur almost exclusively on treatment, the majority in the induction phase of chemotherapy. In a retrospective survey, 90% of symptomatic thrombosis in patients treated on BFM-90 occurred during induction when patients were receiving glucocorticosteroids and Escherichia coli asparaginase (Sutor et al., 1999). In their meta-analysis, Caruso et al. (2006) observed 61 events in 1280 paediatric patients in induction (4.8%, 95% CI: 3.7–6.0) and 12 events in 609 paediatric patients in later phases of treatment (2.0%, 95% CI: 1.1–3.3) (P = 0.004). Although rare, thromboses can occur prior to start of treatment. A retrospective review of 719 consecutive unselected adult patients with acute leukaemia (185 patients with ALL) showed that 2.09% had objectively confirmed, symptomatic venous thrombosis before chemotherapy was started. The age-adjusted risk was increased compared to the general population and the incidence was similar in association with acute myeloid leukaemia and ALL (Ziegler et al., 2005).

Sites of thrombosis

Although both venous and arterial events have been reported, the vast majority are venous. A review of symptomatic cases published between 1966 and 2003, including epidemiological data from childhood ALL trials, found that 73% of all thrombotic events were venous (28% CNS, 45% non-CNS) (Athale & Chan, 2003a). A more recent meta-analysis reported on 91 events; 53.8% were in the CNS, 42.8% non-CNS venous thrombosis and 3.3% an unspecified site (Caruso et al., 2006). However, not all the CNS events were necessarily venous: 26 of 49 CNS events were clearly reported as being venous, the origin of the remainder was less well defined. This meta-analysis determined the risk of a CNS event as 2.9% (95% CI: 2.2–3.8) and non-CNS event 2.3% (95% CI: 1.7–3.2). Publications that included asymptomatic cases, such as the PARKAA study, reported a majority (96.6%) of events in the upper venous system (i.e. above the heart) with only 3.4% of events within the CNS (Male et al., 2003).

Right atrial (RA) thrombosis occurs in 2% of patients with symptomatic thrombosis and is more common in studies where patients are evaluated for asymptomatic events (Athale & Chan, 2003a). Korones et al. (1996) reported 8.8% prevalence of RA thrombi in children with cancer and indwelling catheters, whereas the PAARKA study showed a prevalence of 13.6% in children with ALL (Mitchell et al., 2003a).

The majority of non-CNS events are related to central venous catheters. As indicated above, the incidence of catheter-related thrombosis detected on screening radiography is 30–50% but only a minority are symptomatic (Athale & Chan, 2003a; Male et al., 2003). Glaser et al. (2001) found 50% of children with cancer and implantable ports had evidence of thrombosis when investigated with contrast venography at the
Table I. Selected reports on the incidence of thromboembolic events in patients with acute lymphoblastic leukaemia.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study period, protocol(s), study design</th>
<th>Total number with thrombosis</th>
<th>Incidence/ prevalence (95% CI)</th>
<th>Timing of thrombosis (numbers or %)</th>
<th>Age of thrombotic patients/years Median (range)</th>
<th>Details of events (absolute numbers)</th>
<th>CVL details of patients with thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nowak-Gottl et al (2003). BFM 90/95 data included in reference 2 of this table.</td>
<td>1994–2002 BFM 90/95 BFM 2000 ALL outcome studies</td>
<td>280 29 10 4%</td>
<td>Induction</td>
<td>10 4%</td>
<td>Symptomatic (29)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>2. Nowak-Gottl et al (2001)</td>
<td>1992–1998 BFM 90/95 ALL outcome study</td>
<td>120 3 2 5%</td>
<td>Reinduction</td>
<td>2 5%</td>
<td>Symptomatic (1)</td>
<td>CVL in 100%</td>
<td></td>
</tr>
<tr>
<td>3. Vora et al (unpublished observations)</td>
<td>COALL 92/97 ALL outcome study</td>
<td>1242 36 2 8%</td>
<td>Induction (90%)</td>
<td>2 8%</td>
<td>Symptomatic (36)</td>
<td>CVL-related in 100% of non-CNS</td>
<td></td>
</tr>
<tr>
<td>4. Silverman et al (2001)</td>
<td>UKALL 2003 Ongoing trial</td>
<td>128 9 9–18 years 15%</td>
<td>Intensification</td>
<td>N/A</td>
<td>Included some CNS events (details N/A)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>5. Ku et al (2006)</td>
<td>Registry data</td>
<td>1242 36 2 8%</td>
<td>Induction (90%)</td>
<td>2 8%</td>
<td>Symptomatic (36)</td>
<td>CVL-related in 100% of non-CNS</td>
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</tr>
<tr>
<td>7. Mitchell et al (2003a) PARKAA</td>
<td>CCSG1952,1961 POG9201,9605,9406 Prospectively screened</td>
<td>128 9 9–18 years 15%</td>
<td>Induction (1)</td>
<td>2 7%</td>
<td>Symptomatic (3)</td>
<td>CVL in 100%</td>
<td></td>
</tr>
<tr>
<td>8. Shapiro et al (1993)</td>
<td>BFM-90 Prospective</td>
<td>128 9 9–18 years 15%</td>
<td>Induction (1)</td>
<td>2 7%</td>
<td>Symptomatic (3)</td>
<td>CVL in 100%</td>
<td></td>
</tr>
<tr>
<td>9. Wermes et al (1999a)</td>
<td>BFM-95 Prospective</td>
<td>128 9 9–18 years 15%</td>
<td>Induction (1)</td>
<td>2 7%</td>
<td>Symptomatic (3)</td>
<td>CVL in 100%</td>
<td></td>
</tr>
<tr>
<td>10. Shapiro et al (1993)</td>
<td>CCSG 104,105,123,134,144 Prospective</td>
<td>128 9 9–18 years 15%</td>
<td>Induction (1)</td>
<td>2 7%</td>
<td>Symptomatic (3)</td>
<td>CVL in 100%</td>
<td></td>
</tr>
<tr>
<td>11. Mitchell et al (1994)</td>
<td>DFCI 87-001 Prospective</td>
<td>128 9 9–18 years 15%</td>
<td>Induction (1)</td>
<td>2 7%</td>
<td>Symptomatic (3)</td>
<td>CVL in 100%</td>
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<td>Reference</td>
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<td>12. Caruso et al, 2006 (included data from references 1, 2, 7, 9, 10, 11 in this table)</td>
<td>Meta-analysis of prospective studies published 1986–2003</td>
<td>1752</td>
<td>91</td>
<td>5-2% (4-2–6-4). Induction 48%(3-7–6-0). Post induction 2-0% (1-1–3-3)</td>
<td>5-5</td>
<td>All symptomatic 39/91 non-CNS venous 26/91 SVT 26/91 N/A</td>
<td>25/39 of non CNS events CVL-related</td>
</tr>
<tr>
<td>13. Gugliotta et al (1992)</td>
<td>GIMEMA ALL 0288 Retrospective</td>
<td>238</td>
<td>10</td>
<td>4-2%</td>
<td>Induction</td>
<td>29(12–68)</td>
<td>CNS (7) (5 venous, 2 arterial)</td>
</tr>
<tr>
<td>15. Sutor et al (1999)</td>
<td>BFM-90 1991–93 Retrospective survey</td>
<td>1100</td>
<td>19</td>
<td>1-7%</td>
<td>90% of symptomatic events during induction</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukaemia; UKALL, United Kingdom Medical Research Council/National Research Network Childhood Leukaemia Working Party; BFM, Berlin–Frankfurt–Munster; COALL, German Co-operative Study Group for Childhood Acute Lymphoblastic Leukemia; DFCI, Dana Farber Cancer Institute; CCSG, Children’s Cancer Study Group; POG, Pediatric Oncology Group; PARKAA, Prophylactic Antithrombin Replacement in Kids with ALL on Asparaginase; GIMEMA, Gruppo Italiano Malattie Ematologiche Maligne dell’Adulto; N/A, not available; CI, confidence interval; CNS, central nervous system; CVL, central venous line; DVT, deep vein thrombosis; SVT, cerebral sino-venous thrombosis; LL, lower limb; RA, right atrium; PE, pulmonary embolism; upper venous includes internal jugular, brachiocephalic, subclavian veins and superior vena cava.
site of catheter placement, of which 25% were symptomatic. Similarly, 34% (29/85) of children in the PARKAA study with an upper limb CVL had evidence of thrombosis on radiological screening during induction, of which 83% were at the site of entry of catheter into the vein and the vast majority were asymptomatic (Male et al, 2003).

Risk factors

Thrombotic events occur due to a combination of disease-, host- and treatment-related risk factors. By understanding the relative contribution of individual risk factors in their pathogenesis, preventative strategies can be designed to target those at greatest risk.

Idiopathic venous thrombosis in childhood is rare. A Canadian registry of 137 patients under 18 years found a minority (4%) of thromboses in children were idiopathic (Andrew et al, 1994). A UK survey conducted by the British Paediatric Surveillance Unit (BPSU) between 2001 and 2003 similarly found that only 17 of 172 (10%) cases had no risk factors (Gibson & Bolton-Maggs, 2003). In the latter survey, the main risk factors identified were sepsis, immobility or both (60%), CVLs (48%) and malignancy (26%). A family history of thrombosis was noted in just 10% of cases (E. Chalmers, personal communication of BPSU data presented at BSH 2006).

Apart from a much higher risk in neonates, it is unclear if age is a risk factor. Data from children treated on the DFCI 91-01 study showed that older children (9–18 years) had a higher incidence of thrombosis than younger children (15% vs. 2%; P < 0.01) (Silverman et al, 2001). Athale et al recently reported that older age was associated with an increased risk of thrombotic events in children treated on more recent Dana-Farber Cancer Institute (DFCI)-ALL protocols (DFCI 95-01 and 2000–01). However, high risk disease was also associated with a higher risk of thrombosis, so age-related risk may partly reflect the effect of ALL-risk stratification and more intensive treatment (Athale et al, 2005). The importance of age as an independent risk factor for thrombosis in childhood ALL was not evident in the other studies reviewed here. There is no demonstrable effect of gender on development of thrombosis.

Disease

Several studies have documented increased thrombin generation at diagnosis which persists for several months after starting therapy (Priest et al, 1982; Rodeghiero et al, 1990; Mitchell et al, 1994; Oner et al, 1999; Giordano et al, 2000; Uszynski et al, 2000). At presentation, markers of in vivo thrombin generation are increased compared with healthy controls (Mitchell et al, 1994). Malignant cells may alter haemostasis in a number of ways. Procoagulant molecules and inflammatory cytokines may be directly synthesised by cancer cells. In addition, malignant cells can interact with vascular endothelial cells to produce a prothrombotic state (Rickles & Falanga, 2001; Sutherland et al, 2003) by compromising their anti-coagulant properties and increasing the release of procoagulant proteins such as factor VIII (FVIII) from stores.

Treatment

The importance of treatment in the pathogenesis of ALL-related thrombosis is highlighted by the observation that, unlike in adult solid tumours, thrombosis rarely occurs at diagnosis. Over 90% of events occur during induction, the remaining during consolidation or intensification courses, hence, attention has focused on chemotherapeutic agents given during these phases for their influence on haemostasis. The most extensively studied are asparaginase and steroids (Athale & Chan, 2003b).

Asparaginase. Asparaginase is an important component of induction and intensification therapy. The cytotoxicity of asparaginase is mediated by depletion of the essential amino acid asparagine. In comparison with normal cells, lymphoblasts lack the enzyme asparagine synthetase, which makes them particularly susceptible. Asparaginase also reduces circulating levels of several haemostatic proteins including plasminogen, fibrinogen and antithrombin by a combination of reduced hepatic production (Bushman et al, 2000) and increased clearance (Mitchell et al, 1995). The degree of deficiency is proportional to serum asparaginase activity and asparagine depletion (Capizzi et al, 1971; Ramsay et al, 1977; Cairo, 1982; Pui et al, 1985, 1986; Bezaud et al, 1986; Miniero et al, 1986; Semeraro et al, 1990; Vigano’D’Angelo et al, 1990; Mitchell et al, 1994, 1995; Muller & Boos, 1998). Deficiency of proteins C and S and qualitative abnormalities of von Willebrand factor (VWF) have also been reported (Pui et al, 1985, 1986; Shapiro et al, 1993; Mitchell et al, 1994, 1995), but not consistently (Leone et al, 1993). These anti-coagulant deficiencies result in impaired thrombin inhibition (Mitchell et al, 1994) as detected by an abnormal thromboelastogram (Miniero et al, 1986). Ineffective regulation of excess thrombin has therefore been proposed as the main pathogenetic mechanism for the risk of thrombosis associated with ALL (Mitchell et al, 1995).

Several asparaginase preparations are available, derived from either E. coli strains or Erwinia chrysanthemi (Erwinase). Contradictory results have been obtained on investigation of the haemostatic effects of different asparaginases. Some studies have found a less pronounced effect on coagulation proteins with Erwinase than E. coli-derived asparaginase (Carlsson et al, 1995; Mitchell et al, 1995; Nowak-Gottl et al, 1996a, 1997; Appel et al, 2006). Others report the converse (Albersen et al, 2001) or no difference (Castaman & Rodeghiero, 1993) in the effect of the two preparations. At equivalent dosage, Erwinase produces significantly lower serum asparaginase activity and asparaginase depletion (Duval et al, 2002) than E. coli asparaginase, possibly explaining the less pronounced effect of Erwinase on coagulation observed in some studies. On the
other hand, although length of exposure to asparaginase correlates with thrombosis risk, higher doses do not. On the contrary, there were more thrombotic events in the group receiving ≤6000 U/m² compared with ≥10 000 U/m² in a large meta-analysis (Caruso et al, 2006).

**Steroids.** Glucocorticosteroids are an important component of the treatment of ALL and are frequently given concurrently with asparaginase for remission induction. Prednisolone leads to elevation of factor VIII, VWF, prothrombin and antithrombin levels (Ozsoylu et al, 1982; Isacson, 1970; Dal Bo Zanon et al, 1982; Ueda, 1990). Additionally, it produces a dose-dependent hypofibrinolytic state with an increase in plasminogen activator inhibitor-1 levels demonstrated in human and animal studies (Isacson, 1970; Patrassi et al, 1995). Thomas et al (1993) showed that prednisolone (60 mg/m²/d) led to reduction in VWF antigen (VWF:Ag) and collagen binding activity (VWF:CB) whereas the combination of prednisolone and asparaginase led to an increase in VWF:Ag and VWF:CB as well as an increase in large molecular weight multimers of VWF.

Although there are no randomised studies comparing the haemostatic effects and thrombosis risk associated with different glucocorticosteroids, a historical comparison of two BFM studies indicated that the risk during induction was much lower with dexamethasone than prednisolone (BFM 2000 – dexamethasone = 1.8%; BFM 90/95 prednisolone = 10.4%, P = 0.028) (Nowak-Gottl et al, 2003). However, the groups were not directly comparable, with differences in the number of patients at risk and duration of steroid exposure (Athale & Chan, 2003b). A meta-analysis of prospective studies showed no statistically significant difference in thrombosis incidence according to the glucocorticosteroid type used in induction (although numbers receiving dexamethasone were small) but found instead that prednisolone was associated with a higher risk in post-induction phases (12.2% vs. 1.6%, P = 0.001) (Caruso et al, 2006).

**Central venous lines**

Central venous lines are the most frequent cause of thrombosis in children, accounting for more than two-thirds of events (Massicotte et al, 1998). Children with ALL are more likely to have CVL-related thromboses than children with other malignancies (Korones et al, 1996; Werms et al, 1999a). The majority of CVL-related thromboses are asymptomatic and most frequently at the entry site of the catheter into the vein (Male et al, 2003). A recent meta-analysis reported that a majority of non-CNS events were catheter-related (Caruso et al, 2006). CVL-related thrombosis not only compromises effective delivery of treatment but can also lead to additional problems, such as recurrence of thrombosis (4–19%), post-thrombotic syndrome (5–25%) pulmonary embolism (8–15%) and death (2–4%) (Massicotte et al, 1998; Glaser et al, 2001)

A PARKAA substudy investigated the risk factors for CVL-related thrombosis and found the risk to be higher with left-sided catheters (OR 2.5, 95% CI: 1.0–6.4, P = 0.048), subclavian insertion (OR 3.1, 95% CI: 1.2–8.5, P = 0.025) and when inserted by percutaneous techniques compared with venous cutdown (OR 3.5, 95% CI: 1.3–9.2, P = 0.011). A left-subclavian CVL is presumed to cause greatest reduction to venous flow as the line follows a longer course and enters the SVC at a sharper angle. Percutaneous techniques may be associated with increased venous trauma than surgical cutdown (Male et al, 2003).

In a retrospective analysis of patients with standard risk ALL enrolled in Paediatric Oncology Group (POG) 9201 trial, external lines were associated with a higher risk of thrombosis than fully implanted CVLs (port-a-caths) (odds ratio 3.9, 95% CI: 1.5–10.3, P = 0.006) and were more likely to be removed (for any reason) (OR 5.6, 95% CI: 2.7–12.1, P < 0.001). Early CVL placement (prior to day 15 of induction) was not a risk factor for thrombosis or CVL removal (McLean et al, 2005) but was associated with an increased risk of infection.

**Inherited thrombophilia**

Inherited thrombophilia influences an individual patient’s risk of developing thrombosis under environmental prothrombotic stress and may be useful for designing targeted primary or secondary prevention strategies. Unfortunately, although no doubt contributing to an increased risk, it is not entirely clear how screening for inherited thrombophilia should guide strategies for the prevention and treatment of thrombosis in childhood (Nowak-Gottl et al, 1996b; Revel-Vilk et al, 2003). In the context of ALL, the literature indicates that inherited thrombophilia does contribute to an increased risk. A multicentre, prospective study of patients treated on BFM 90/95 evaluated the risk of thromboembolism in children who had at least one identifiable prothrombotic defect at diagnosis. It found that the risk was higher in patients with a prothrombotic defect (46.5% vs. 2.2%, P < 0.0001) and was cumulative; patients with multiple defects had a significantly higher risk compared to those with a single defect (P = 0.009) (Nowak-Gottl et al, 1999). Protein C, S and AT deficiency were associated with the highest risk. Other smaller studies have reported similar results (Wermes et al, 1999a,b). In contrast to these studies, the North American PARKAA study failed to show any correlation between the presence of FV Leiden, prothrombin mutation 20210A and the development of thrombosis (Mitchell et al, 2003a). However, patient numbers were small and the study was underpowered to detect a statistically significant association. Also, as these defects are generally regarded as lower risk for thrombosis than Protein C, S and AT deficiency (which were not assessed in the PARKAA study) it is perhaps not surprising that an association was not found. A meta-analysis concluded that inherited thrombophilia increased the thrombotic risk in ALL patients by approximately eightfold (Caruso et al, 2006).
Interaction between risk factors

A relatively low thrombotic risk associated with individual drugs or an inherited defect is amplified in combination with other risk factors. Used separately, asparaginase and steroids have a low risk of thrombosis but in combination the risk rises nearly 10-fold as demonstrated by a retrospective comparison of the risk of symptomatic thrombosis in contemporaneously treated German patients on the Cooperative Acute Lymphoblastic Leukaemia (COALL) 92/97 protocols (1.5%) and those treated on BFM 90/95 (11%) (Mauz-Korholz et al, 1999). A multi-centre prospective study of 420 consecutive patients (BFM n = 300, COALL n = 120) subsequently confirmed these results (BFM = 11.6%, COALL = 2.5%, OR = 7.7, P = 0.0005) (Nowak-Gottl et al, 2001). In both protocols, thrombotic episodes occurred exclusively during asparaginase exposure, but the difference in incidence was due to the timing of exposure. Asparaginase is administered from early induction, concurrent with steroids, in BFM protocols, whereas in the COALL regime it is administered during consolidation therapy when patients are not receiving steroids. A later BFM study indicated that the incidence was lower when dexamethasone is given in combination with asparaginase compared with prednisolone (Nowak-Gottl et al, 2003).

Protocol differences also influence the risk associated with inherited thrombophilia (Mauz-Korholz et al, 2000; Nowak-Gottl et al, 2001). Although the prevalence of prothrombotic defects was similar in the populations at risk, patients with thrombosis had a higher prevalence of defects in the BFM but not the COALL study (Mauz-Korholz et al, 2000). For the combined population, only concurrent administration of E. coli asparaginase and prednisolone in children with a prothrombotic risk factor was found to increase the risk of a thrombotic event (odds ratio 34.5, 95% CI: 4.39–271.42; P = 0.0008) (Nowak-Gottl et al, 2001).

In conclusion, patients with one or more inherited prothrombotic defects (especially protein C, S or AT deficiency) receiving a combination of E. coli asparaginase and prednisolone during induction therapy appear to be at highest risk of thrombosis.

Morbidity and mortality

National and international registries have provided evidence of the not insignificant morbidity (16% incidence of pulmonary embolism in CVL-related thrombosis) and mortality (1.5–2.0%) associated with thrombosis in children overall (Andrew et al, 1994; van Ommen et al, 2001; Gibson & Bolton-Maggs, 2003). Morbidity resulting from thrombosis in ALL is less well-documented, as registries report global events in children and few large ALL focused studies have sufficient follow-up data to be informative. What little data are available indicate that immediate thrombosis-related mortality is extremely low. Although patients with CNS thrombosis often present with major neurological deficits, full neurological recovery is the norm. Loss of a CVL due to thrombosis often compromises the delivery of chemotherapy and intravenous supportive care. The short-term consequences, therefore, are suboptimal delivery of treatment or prolonged interruptions; both with possible impact on long term survival (Ogawa et al, 2005). Post-thrombotic syndrome (PTS), a clinical syndrome of pain, swelling and skin changes in a limb previously affected with venous thrombosis, has been reported in 9–12.4% (Massicotte et al, 1998; van Ommen et al, 2001; Kuhle et al, 2003) of children after DVT, but is unlikely to be that frequent after CVL-related upper venous thrombosis in the context of ALL. The morbidity associated with asymptomatic CVL-related thrombosis found on routine screening is unknown.

Prevention

Given the significant burden of thrombotic complications, primary prophylaxis would appear to be warranted in high-risk groups during asparaginase exposure. However, there is as yet insufficient evidence on the safety and efficacy of anticoagulant prophylaxis to justify their routine use, even for high-risk groups.

Whether anti-coagulation is justified in a particular prothrombotic condition is determined by a calculation of bleeding risk of the intervention set against the reduction in risk of thrombosis. Unfortunately, studies of thrombosis risk in ALL rarely report bleeding complications associated with anti-coagulant prophylaxis or treatment. In a meta-analysis of 17 prospective studies, only 11 provided data on haemorrhagic complications (Caruso et al, 2006); the pooled incidence of bleeding was 2% and thrombosis risk 5.2%. Furthermore, there is no convincing evidence that either anti-coagulation or replacement therapy reduces the risk of thrombosis.

Prophylactic anti-coagulation with low-dose warfarin or enoxaparin was not effective in reducing the risk of CVL-related thrombosis in adults in placebo-controlled, randomised trials (Couban et al, 2005; Verso et al, 2005). Similarly, a randomised, placebo-controlled study of children with malignancies and CVLs did not show any reduction in the incidence of CVL-related thromboses with the use of low dose warfarin (target International Normalised Ratio 1.3–1.9) (Rued et al, 2006). Overall, studies in adults have given contradictory results with use of low molecular weight heparin (LMWH) (Monreal et al, 1996) or fixed low-dose warfarin (Bern et al, 1990), (Mismetti et al, 2003) such that current guidelines do not recommend their use (Geerts et al, 2004).

A multicentre randomised trial of LMWH for the prevention of CVL-related thrombosis in children with a variety of disorders (including ALL), the PROphylaxis of ThromboEmbolism in KidsTrial (PROTEKT), allocated 186 children with a new CVL to receive either LMWH or standard care until the CVL was removed, or for 30 d, and evaluated the incidence of thrombosis with exit venograms. There was no difference in CVL-related thrombosis in the two arms (13%) but the study was underpowered because of premature closure due to slow
recruitment (Massicotte *et al*., 2003a). The current American College of Chest Physicians (ACCP) anti-coagulation and thrombolytic guidelines for children advise against routine primary prophylaxis for children with CVLs (Monagle *et al*., 2004). However, the issue remains under investigation. In a non-randomised cohort of 41 children with ALL who received enoxaparin, there were no thrombotic events or bleeding episodes (Elhasid *et al*., 2001) and the safety and efficacy of LMWH for prevention of thrombosis during induction chemotherapy is being evaluated in an ongoing multicentre trial – the THROMBOTECT trial (Greiner & Korte, 2004).

Heparins require normal AT levels for optimal anti-coagulant effect and, therefore, may not be consistently effective during the period of asparaginase-induced AT deficiency. New direct thrombin inhibitors do not rely on AT for inhibition of thrombin and a recent in *vitro* study demonstrated that the direct thrombin inhibitor, melagatran, produced a consistent reduction in endogenous thrombin generation capacity, independent of endogenous antithrombin levels, in plasma from children with ALL taken during induction. By contrast, the anticoagulant action of LMWH was markedly affected by endogenous AT levels. There was a direct relationship between the anticoagulant effect of LMWH and AT concentration (Kuhle *et al*., 2006). Unfortunately, the use of melagatran is associated with hepatic toxicity, such that the drug previously used in clinical trials in adults (ximelagatran) has been withdrawn from the market. However, safer direct thrombin inhibitors (and other novel anticoagulants independent of AT for their effect) may warrant study as prophylactic agents in ALL.

The presumption that a reduction in natural anticoagulants is a major factor in mediating the pathogenesis of thrombosis seen with asparaginase therapy has led several groups to routinely use replacement therapy, either in the form of fresh frozen plasma (FFP) or AT concentrates, during asparaginase exposure. The most recent UK adult ALL trial (UKALLXII) (http://www.ctsu.ox.ac.uk/projects/leuk/ukallxii/) recommended monitoring of fibrinogen, AT and/or PTT during asparaginase exposure and advocated administration of FFP if these parameters were markedly deranged. However, the evidence for such an approach is lacking. FFP is ineffective in correcting the coagulant deficiencies or normalising markers of endogenous thrombin generation in children with ALL receiving asparaginase (Halton *et al*., 1994; Nowak-Gottl *et al*., 1995). Moreover, the risks associated with liberal use of FFP are increasingly recognised (O’Shaughnessy *et al*., 2004).

AT concentrates, given at appropriate doses and frequency, can correct the hypercoagulability associated with asparaginase therapy (Gugliotta *et al*., 1990) but their clinical benefit is uncertain. Retrospective studies have reported inconsistent results. Elliott *et al*., 2004) observed fewer thrombotic events in supplemented adults but Hongo *et al*., 2002) were unable to demonstrate any benefit of AT supplementation in children. Prospective studies have been equally inconclusive. Nowak-Gottl *et al*., 1996c) found that AT supplementation corrected coagulation parameters but the sample size was not large enough to demonstrate clinical efficacy in children treated on the ALL–BFM-90 protocol. A prospective, randomised, controlled phase II trial of AT replacements therapy in children (PARKAA) was underpowered to test the clinical efficacy of AT concentrate but showed a trend towards efficacy and safety of AT concentrate (Mitchell *et al*., 2003b). In addition, unlike previous trials, it failed to show any difference in markers of thrombin generation in supplemented children. Thus, larger studies are required to answer this question conclusively. It is hoped the ongoing German THROMBOTECT trial will inform the development of risk-adapted prophylaxis guidelines (Greiner & Korte, 2004).

There are, however, measures not involving the use of anticoagulants that can be effective in reducing the risk of thrombosis. As indicated before, there is evidence that insertion of port-a-caths sited on the right using a cut-down approach for sub-clavian insertion is associated with a lower risk of thrombosis (and infection) compared with external lines (McLean *et al*., 2005), left-sided placement and insertion by a percutaneous approach (Male *et al*., 2003). Although not proven to reduce the risk of thrombosis in a randomised study, as a majority of CVL-related events occur during induction, the recommendation in the UK is to delay insertion, if possible, until the end of that course. Finally, the available evidence indicates that dexamethasone should be the steroid of choice in treatment of ALL, both for its greater anti-leukaemic efficacy compared with prednisolone (Mitchell *et al*., 2005) but also lower thrombotic risk in combination with asparaginase.

**Investigation and management**

**CVL-related thrombosis**

Central venous line-related thrombosis may present with line malfunction or prominent chest wall veins depending on whether the problem is a small clot at the tip, a fibrin sheath forming a valvar occlusion or an extensive DVT (Fig 1). Small asymptomatic clots are often found at the catheter entry site on routine screening and can probably be left untreated. Line malfunction may vary from an inability to draw blood from a line that flushes normally through ‘stiffness’ on flushing to complete occlusion. In the latter circumstance, thrombosis must be distinguished from mechanical failure as a result of incorrect position, fracture or perforation.

A proposed scheme for the investigation and management of CVL occlusion with or without venous thrombosis is described in Fig 2. The extent and urgency of investigations depend on the degree of catheter occlusion and whether a venous thrombosis is suspected. Starting with a chest x-ray to exclude mechanical failure, further imaging may include a linogram (injection of contrast through line under x-ray imaging), bilateral upper limb venography, Doppler ultrasonography (USG) and magnetic resonance venography (MRV). Linograms are relatively insensitive for detection of thromboses and merely exclude the presence of line tip clot or fibrin
sheath. Although venography is the accepted standard for diagnosis of thrombosis, ultrasound was found to be the most commonly used test for suspected thrombosis in the Canadian population-based paediatric registry (Andrew et al, 1994). The advantages of ultrasound include availability, speed and non-invasiveness. In adults with lower limb DVT, ultrasound findings of non-compressibility have excellent sensitivity and specificity for proximal thromboses. However, most thromboses in children occur in the upper venous system in which the reliability of doppler ultrasound has not been widely assessed. In the PARKAA study, venography was more sensitive and specific for detecting thromboses than ultrasonography (sensitivity = 79% vs. 37%, specificity = 92% vs. 79%). Ultrasound was particularly insensitive for thromboses in central veins of the upper venous system, where venography was clearly superior. However, ultrasound was more sensitive for thromboses in the jugular veins where venography was less useful (Male et al, 2002). In practice, most institutions use ultrasound as the initial screening test followed by standard venography or MRV in patients with normal ultrasound who are strongly suspected of having a thrombosis.

Whether to remove the CVL or not depends on its functional status and the continuing need for central access. A completely occluded catheter unresponsive to thrombolytic treatment must be removed. A line associated with an extensive thrombosis is best removed if the line is not essential to the delivery of leukaemia therapy. Most partially occluded CVLs, and even some with complete occlusion, can have patency restored with urokinase or tissue Plasminogen Activator (t-PA) locks (Ponec et al, 2001). The latter have been shown to be more effective than the former in a randomised study (Haire et al, 1994). Infusions of urokinase or t-PA at very slow infusion rates are sometimes effective in restoring patency to even completely occluded lumens (Bagnall et al, 1989; Haire et al, 1990) (see Fig 2 for dose recommendations). We recommend that extensive venous thrombosis is managed with 1–3 months of treatment dose anticoagulation with LMWH (twice daily to maintain a 4-h post-dose anti-Xa level of 0.5–1.0 IU/ml) followed by prophylactic doses (once daily to maintain anti-Xa level of 0.1–0.3 IU/ml) during asparaginase therapy until the CVL is removed as described below. In the event of symptomatic recurrence despite prophylactic anticoagulation, treatment doses are recommended until CVL removal. Our personal experience is that, if required, a CVL can be re-inserted into a different vein without incurring a significant risk of recurrent thrombosis.

CNS thromboses

Cerebral sino-venous thrombosis (SVT) may present with headache, seizures, focal neurological deficit or loss of consciousness. Although often the initial investigation, a computed tomography (CT) scan is not as sensitive as MRI or MR angiography (Fig 3) (Purvin et al, 1987; Nicholson et al, 1996; Corso et al, 1997) and the latter is the investigation of choice for diagnosis of SVT.

A proposed scheme for management is described in Fig 4. A Cochrane review of available evidence regarding the effectiveness and safety of anticoagulant therapy included two small, unconfounded randomised trials, which together entered only 79 patients. One trial examined the use of unfractionated heparin and the other LMWH. The authors concluded that anticoagulant treatment appeared to be safe and was associated with a potentially important reduction in the risk of death or dependency but this did not reach statistical significance (Stam et al, 2002). Another Cochrane review did not identify any randomised trials showing benefit of thrombolysis in SVT (Ciccone et al, 2004). The ACCP guidelines for children recommend anticoagulation unless there is a major CNS haemorrhage. A period of 3 months of anticoagulation is suggested, extending to 6 months if full re-canalisation is not seen on follow-up CT or MRV scan (Monagle et al, 2004). A week of initial anticoagulation with unfractionated heparin or LMWH followed by oral anticoagulation is recommended. A large multicentre retrospective study of ischaemic stroke in children with ALL identified 11 cases of SVT in 2318 patients (0.47% prevalence). Seven of 11 patients received anticoagulation including two patients with co-existent cerebral haemorrhage. Antithrombotic therapy in this small group of patients was not associated with excessive bleeding complications (Santoro et al, 2005).

Currently, the majority of childhood ALL study groups recommend anti-coagulation with or without AT concentrates for initial treatment of SVT. Patients can be re-exposed to asparaginase under cover of anti-coagulant prophylaxis (LMWH, anti-Xa level 0.1–0.3 IU/ml) without risk of recurrence. Some groups also recommend AT concentrates for secondary prophylaxis.
Anti-coagulant therapy

Readers are directed to the ACCP guidelines 2004 for comprehensive recommendations on choice of anti-coagulant therapy, doses and monitoring (Buller et al, 2004; Hirsh & Raschke, 2004; Monagle et al, 2004). More recent guidelines on oral anticoagulation and heparin use in adults are available from the British Committee for Standards in Haematology (BSCH) (Baglin et al, 2006). The efficacy and safety of anticoagulation in patients with leukaemia has been demonstrated in several series (Mauz-Korholz et al, 1999; Imberti et al, 2004; Ziegler et al, 2005). In a retrospective study of 719 leukaemic patients, 14 of 15 patients with a thrombosis were anti-coagulated without major bleeding problems (Ziegler et al, 2005). LMWH (doses as above) is the heparin of choice as it has a number of advantages over unfractionated heparin (Hirsh & Raschke, 2004; Baglin et al, 2006), including subcutaneous administration and predictable pharmacokinetics requiring less frequent monitoring and allowing out-patient treatment. In addition, LMWH is associated with a lower risk of heparin-induced thrombocytopenia and osteoporosis. The safety and efficacy of LMWH compared with unfractionated heparin followed by oral anticoagulation was examined in the REVIVE (Reviparin in childhood venous thromboembolism) study (Massicotte et al, 2006).

Fig 2. Management of central venous line (CVL)-related thrombosis. LMWH, low molecular weight heparin; DVT, deep vein thrombosis; MRV, magnetic resonance venography.
REVIVE was the first multicentre, international randomised controlled trial to compare these approaches to anticoagulation for the treatment of childhood venous thrombosis and included childhood cancer patients. Unfortunately the study was underpowered to test efficacy due to premature closure because of slow patient recruitment. However, the study showed that in children >3 months, treatment with LMWH was as effective and safe as unfractionated heparin.

ACCP guidelines recommend 3 months of anticoagulation for children beyond the neonatal period with secondary thrombosis (Monagle et al., 2004). To avoid the problem of drug interaction, we recommend treatment with twice daily LMWH (anti-Xa levels 0.5–1.0 IU/ml) over warfarin in the setting of ALL. Re-exposure to asparaginase should be covered with once daily prophylactic LMWH (anti-Xa level 0.1–0.3 IU/ml) starting the day before the first dose and for 48 h after the last dose (or 2 weeks after pegylated asparaginase). Anticoagulation in patients with severe thrombocytopenia requires careful management to avoid serious bleeding complications. Options include supporting the platelet count with transfusions to maintain a platelet count of at least 30–50 \( \times 10^9/l \) (http://www.ctsu.ox.ac.uk/projects/leuk/ukall2003), or reduction/cessation of heparin (Ziegler et al., 2005) during periods of thrombocytopenia, although only the former strategy can be regarded as delivering full anticoagulation. An additional consideration is the need for frequent lumbar punctures necessitating temporary cessation of anticoagulants. The omission of LMWH for the previous 24 h is generally sufficient.

Thrombophilia screening

Given the conflicting literature on the role of inherited thrombophilia in children with ALL, universal thrombophilia screening is not justified. However, if there is a confirmed family history of one of the higher risk prothrombotic defects, such as AT, protein C or protein S deficiency, directed screening is appropriate since replacement therapy during high risk periods of treatment might be justified in such patients. To determine the relative risk associated with individual defects in the context of specific chemotherapy protocols, ALL study groups may wish to include thrombophilia screening at diagnosis as an integral part of their treatment trials.
Future directions

The studies cited here have provided useful information on epidemiology, risk factors and treatment interventions but suffer from small sample size or selection bias and are, therefore, often inconclusive. To improve the evidence base, large clinical treatment trials should incorporate studies to define risk factors for thrombosis, which would enable the design of targeted primary and secondary prevention strategies. Recombinant AT concentrates and oral direct thrombin inhibitors are in phase III clinical trials and, if found safe, could then be tested for primary prevention in the high-risk groups uncovered by such studies. In view of the relatively low number of events, international collaboration will be needed to accrue the large numbers of patients for intervention trials to be adequately powered.

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References


