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Thrombophilia in Children With Cystic Fibrosis

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Summary. In some children with cystic fibrosis (CF), percutaneous long lines occlude sooner than expected (due to thrombophlebitis or thrombosis), and many have a totally implantable venous access device (TIVAD), a recognized complication of which is thrombosis. This complication is more likely if the child has an underlying thrombotic tendency, which may be enhanced in the presence of inflammatory lung disease. There are no reports of an identified association of heritable thrombophilia with CF, although individual cases have been recognized. Our aim was to determine the incidence of thrombophilia in children with CF. In a tertiary pediatric CF center, blood was screened for thrombophilia at annual review, and retested if abnormal. A thrombotic abnormality was found in 41/204 (20%) patients. These included activated protein C resistance (10/204, 5%) with a prevalence similar to that expected, but the following abnormalities had an increased prevalence: antithrombin deficiency (2/204, 1%), protein S deficiency (11/204, 5%), protein C deficiency (8/204, 4%), and lupus anticoagulant (18/204, 9%). There were no differences found in those with thrombophilia for the following parameters: age, gender, genotype, lung function, presence of *Pseudomonas aeruginosa*, prothrombin time, serum IgE, aspergillus-specific IgE, liver function, and blood inflammatory markers. Fifteen children had TIVADs, 4 of whom had evidence of thrombophilia. In conclusion, a significant proportion of patients had a thrombophilic abnormality. We recommend that thrombophilia screening be performed prior to insertion of a TIVAD, and also in those with a history of venous thrombosis, blocked TIVADs, or recurring problems with long lines. **Pediatr Pulmonol. 2005; 39:306–310.** © 2005 Wiley-Liss, Inc.

Key words: thrombophilia; clotting; cystic fibrosis; children.

INTRODUCTION

Intravenous antibiotics play a critical part in the management of cystic fibrosis (CF) lung disease, and due to the length of the antibiotic courses, they are usually administered by peripherally inserted central catheters. Although anecdotal, there are some children whose percutaneous long lines seem to block sooner than expected, and presumably this is due to superficial thrombophlebitis or venous thrombosis. In addition, many CF patients (23% according to UK CF database 2002) have a totally implantable venous access device (TIVAD) such as a Port-a-Cath®, and a recognized complication (reported in 3–40%) is line occlusion and thrombosis, with the clot often attached to the tip of the catheter.[1–9] These complications are more likely if the child has an underlying thrombotic tendency, and this tendency may be enhanced in the presence of lung infection and inflammation.[10,11] The aim of this study was to screen our pediatric CF clinic to determine the underlying incidence of thrombophilia and see if there was any correlation with clinical status.

Currently there is no accepted international definition of thrombophilia.[12] In 1990, the British Committee for Standards in Haematology defined it as ''familial or acquired disorders of the haemostatic mechanism which are likely to predispose to thrombosis.''[13] However, it is now recognized that many individuals who carry these defects remain asymptomatic, and at least 50% of those with a history of thrombosis have no defect identified.[12] For this reason, some use the term to refer to patients who have a tendency to develop thrombosis,[14] whether spontaneously, recurrently, more severely than expected from other risk factors, or at a relatively young age.[12] For the purposes of this study, we used the original 1990 definition, since we were screening patients who did not necessarily have symptomatic thrombosis. A number of these disorders have been identified, but there are no reports of a particular association of heritable thrombophilia with CF. Individual cases, however, have been recognized in whom venous thrombosis occurred secondary to a

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previously undetected clotting abnormality, sometimes but not always combined with an indwelling venous catheter.[10,15–17] There are also two recent abstracts in which a number of CF patients were screened and found to have a disorder.[18,19]

PATIENTS AND METHODS

The setting was a specialist pediatric CF unit in a London tertiary center. At time of annual review, in addition to the usual protocol (available on http://www.rbh.nthames.nhs.uk/children.cf), blood was assayed in all children for thrombophilia screening, with no extra blood taken. If abnormal, the thrombophilia screen was repeated. The screen (and our reference ranges) included fibrinogen levels (1.5–4.5 g/l), activated protein C resistance (APCR) (2.8–7.0 ratio), free protein S activity (65–130%), protein C activity (70–130 IU/dl), antithrombin activity (70–130 IU/dl), and lupus anticoagulant (0.80–1.10 ratio), using standard laboratory methods.[20,21] Patients with the lupus anticoagulant were also tested for the presence of anti-protein S antibodies.[22] Coagulation times were also measured: prothrombin time (10.2–14 sec), activated partial thromboplastin time (26–36 sec), and thrombin time (9–16 sec). Prothrombin G20210A gene mutation and homocysteine levels were not measured.

Demographic data and results of lung function (forced expiratory volume in 1 sec (FEV_1), using published reference ranges[23]), sputum or cough swab microbiology, and blood testing were then recalled from our CF database. The following blood results were reviewed: liver function, i.e., aspartate transaminase (AST) and γ-glutamyl transferase (γ-GT), and prothrombin time; and blood inflammatory markers: total IgG, erythrocyte sedimentation rate (ESR); and *Aspergillus* markers total IgE and radioallergosorbent test (RAST) for *Aspergillus*-specific IgE.

RESULTS

Thrombophilia screening was performed on 204 patients, and abnormalities were found in 41 (20%) children. On repeat testing, 37/38 were still abnormal, one child previously positive for lupus anticoagulant was later negative, and 3 patients are still to be retested. There were 97 boys (48%) and 107 girls (52%) aged 1–17 years (median 9.0 years). The genotype was ΔF508/ΔF508 in 52.5%, other abnormalities in 34.5%, and undetermined in 13%. No patient had a history of symptomatic thromboembolism. TIVADs were present in 15 (7%) children, 4 of whom had evidence of thrombophilia. CF-related diabetes (CFRD) was diagnosed in 8 (4%) children. No children were taking regular vitamin K supplementation.

A thrombotic abnormality was found in 41/204 (20%) patients (Table 1). These included activated protein C resistance (10/204, 5%), which is similar to the expected rate in the general population.[14] However the following abnormalities were greater than expected: antithrombin deficiency (2/204, 1%), protein S deficiency (11/204, 5%), protein C deficiency (8/204, 4%), and lupus anticoagulant (18/204, 9%).[14,24,25] A combined deficiency was found in 11/204 (5%) children. Two combinations accounted for the majority: lupus anticoagulant with low free protein S (6/11), and lupus anticoagulant with activated protein C resistance (4/11). There was one patient with both low protein C and low free protein S. In addition, 14/18 (78%) children with lupus anticoagulant had anti-protein S antibodies. Fibrinogen levels were all normal. Prothrombin times >14 sec were found in 14 (5%) patients; activated partial thromboplastin time and thrombin time were all normal.

TABLE 1— Clotting Abnormalities (%) in 204 CF Patients Compared With Expected Abnormality Rates From Normal Populations[1]

	CF abnormality rate	Expected population abnormality rate
Prothrombin time	5%	N/A
Fibrinogen	0	N/A
Activated protein C resistance	5%	3–7%
Antithrombin deficiency	1%	0.02%
Protein C deficiency	4%	0.2–0.4%
Protein S deficiency	5%	0.3%
Lupus anticoagulant	9%	1–5%
Lupus anticoagulant + protein S deficiency	3%	Unknown
Lupus anticoagulant + activated protein C resistance	2%	Unknown
Protein C + protein S deficiency	0.5%	Unknown

[1]Abnormality rates for activated protein C resistance, antithrombin deficiency, and protein C deficiency were from Lane et al.[14] protein S deficiency from Carraro et al. [24] and lupus anticoagulant from Petri.[25] N/A, not applicable.

There were no differences found between those with and without thrombophilia in the following parameters: age, gender, CF genotype, presence of CFRD, FEV_1, presence of *Pseudomonas aeruginosa*, prothrombin time, liver function, *Aspergillus* markers, and blood inflammatory markers (Table 2).

DISCUSSION

We found an unusually high prevalence of thrombophilic abnormalities in our pediatric CF population (20%), although the prevalence of activated protein C resistance was as expected.[12,14,24,25] It is unclear why the prevalence was so high, and although it is unlikely that it is associated with the basic CF transmembrane conductance regulator (CFTR) defect, this has not been studied. The abnormalities were genuine rather than transiently acquired, since they persisted when retested, except for one child with lupus anticoagulant. Transient lupus anticoagulants are

TABLE 2— Demographic Data and Results in Those With and Without Thrombophilic Abnormality[1]

		Thrombophilic abnormality (n = 41)	No abnormality (n = 163)
Age	Range (years)	1.1–17.0	1.0–17.4
	Median (years)	9.0	9.0
Gender	Male	21 (51%)	76 (47%)
	Female	20 (49%)	87 (53%)
Genotype	ΔF508/ΔF508	20 (48%)	87 (53%)
	Other abnormalities	14 (34%)	56 (34%)
	Undetermined	7 (17%)	20 (12%)
CFRD	Number	2 (5%)	6 (4%)
FEV_1	Median (% predicted)	92%	91%
Pseudomonas aeruginosa	Present	25 (61%)	104 (64%)
Prothrombin time	Abnormal (>14 secs)	4 (10%)	7 (4%)
AST	Abnormal (>50 IU/l)	1 (3%)	4 (3%)
γ-GT	Abnormal (>40 IU/l)	0 (0%)	5 (3%)
IgE	>500 IU/ml	2 (5%)	22 (13%)
Aspergillus RAST	Abnormal (>0.34 IU/ml)	16 (39%)	65 (40%)
IgG	Mean (g/l)	9.3	8.8
ESR	Abnormal (>10 mm/hr)	10 (24%)	41 (25%)

[1]Numbers are given with % in parentheses when appropriate.

common and were detected in up to 30% of children after viral infections.[26] Most of these are not associated with thrombosis except those occurring postvaricella, where life-threatening thrombosis can occur with acquired protein S deficiency and purpura fulminans.[26] It is critical that acquired abnormalities be excluded by follow-up testing, especially in children with concomitant disorders, as transient deficiencies are frequently found.[27] In CF children, several factors might increase the risk of thrombotic abnormalities, the most important of which is the presence of an indwelling central line.[27] Catheters are thrombogenic because they contain foreign material, damage vessel walls, and disrupt blood flow, and in addition some of the infused substances can affect vessel walls.[28] There are many other risk factors for thrombophilia. Those most relevant to CF are liver disease, diabetes, vitamin K deficiency, and immobility.[12,21,29] We found an equal prevalence of liver abnormalities and diabetes in those with and without thrombophilic abnormalities. Vitamin K deficiency is associated with acquired protein S and protein C deficiency,[29] and low vitamin K levels are common in children with CF, even in those with pancreatic sufficiency.[30] None of our patients were on vitamin K supplements at the time, so it is possible that low vitamin K levels were responsible for some of the abnormalities seen, although only two patients with a prolonged prothrombin time also had a concomitant low protein C. Since early 2003, it has been our policy to prescribe vitamin K supplements for all children over age 6 years, to promote healthy bone formation and hopefully reduce the incidence of future osteoporosis, which is becoming increasingly common in CF adults.

Lung infection and inflammation, which are common if not inevitable in most CF patients, can also promote thrombophilia. Protein S exists in either a functionally active form or an inactive form bound to C4b-binding protein.[31] This latter protein is an acute-phase reactant increased in the presence of infection or inflammation; hence the ratio of active to inactive forms of protein S will be adversely affected by an increase in available C4b-binding protein.[10] Functional protein S was also shown to be negatively associated with winter respiratory infections.[11] It is unlikely that this was a major factor with our patients, however, as the group with thrombophilia was not obviously more affected by lung inflammation and infection. Although only a crude marker of inflammation (particularly compared to bronchoalveolar lavage analysis), we found the serum IgG and ESR to be similar in both groups. Lung function and prevalence of *Pseudomonas aeruginosa* were also equal in both groups. Finally, blood was taken at outpatient annual review, so it can be assumed that most patients were clinically stable at the time, although undoubtedly some may have been suffering from a chest exacerbation.

The importance of detecting a thrombophilic abnormality lies in the increased risk of venous thromboembolism that an abnormality may carry, especially in the presence of additional risk factors (accepting that many ''affected'' individuals remain asymptomatic). The relative risks in adults are not well-established, with varying figures quoted, but it is generally accepted that they range from antithrombin deficiency (the most severe) to protein C and S deficiencies (intermediate severity) to activated protein C resistance (the least severe).[20] In children, however,

borderline low concentrations of antithrombin and protein C may not be of such significance, as it is thought that the thrombin inhibitor α-2-macroglobulin, which remains raised throughout childhood, may compensate for the lower antithrombin and protein C concentrations. Lupus anticoagulants are a subset of anti-phospholipid autoantibodies (together with anti-cardiolipin antibodies) and are thrombogenic.[29] The role of these abnormalities in childhood thrombosis is even harder to establish, because although a number of case reports associate antithrombin, protein C, and protein S deficiencies with childhood thrombosis, only a small number of larger case-control studies have identified these defects as independent risk factors.[27] More data are available on activated protein C resistance, presumably due to its higher prevalence in the general population, and although again there are problems interpreting some of the data, it would seem to be a significant risk factor for thrombosis.[27] Of the 10 patients found to have activated protein C resistance, 3 were confirmed as heterozygous for the factor V Leiden defect, but the other 7 did not have DNA analysis performed. Nevertheless, the current method for testing activated protein C resistance in this institution is 100% sensitive and specific for the factor V Leiden defect. The evidence is less convincing for the prothrombin G20210A mutation, and measurement of homocysteine was included in very few childhood studies. In fact, we recently identified an adolescent with CF who has hyperhomocysteinemia and has had problems with long lines for some time. The combination of two thrombophilic abnormalities appears to multiply the thrombotic risk, but large studies are needed to define interactions between several risk factors.[32] The presence of protein C or S deficiency with activated protein C resistance carries a higher risk of thromboembolism than either defect alone, and individuals with antithrombin deficiency combined with activated protein C resistance are likely to develop thromboembolism at a younger age than those with antithrombin deficiency alone.[20] In our CF population, 5% of patients had a combined deficiency, almost all of which included the lupus anticoagulant. It was reported that an association of lupus anticoagulant with anti-protein S antibodies leads to a significant risk of deep vein thrombosis (DVT) in adults.[22] The thrombotic risk of anti-protein S antibodies is unclear, and the finding that 78% of CF children with lupus anticoagulant had anti-protein S antibodies (with potential acquired free protein S deficiency) needs further clarification.

Although we detected an abnormally high prevalence of thrombophilic abnormalities in our CF pediatric population, we are not suggesting that every CF patient should be screened. The consensus is that routine screening of asymptomatic children is not recommended,[24,27] and although CF patients would seem to be at increased risk, there is not enough evidence to recommend testing them all. Nevertheless, some CF units may choose to do so, and although it is relatively easy to do this at annual review, it is important that a protocol is in place for dealing with abnormal results and counseling the patient/family.[12] This should only be undertaken in conjunction with a hematology department. We certainly recommend that testing be carried out in any patient with a history of venous thromboembolism (although not in the acute stage[32]), in those who have recurring problems with long lines, and in those who have had a blocked TIVAD (presumably due to thrombosis). We also recommend preoperative testing for all patients who are due to have a TIVAD inserted. Detecting an abnormality would not prevent the procedure from being carried out; nor would anticoagulant therapy necessarily be initiated. However, part of the informed consent should then include the fact that there is a greater risk for that individual of complications with the TIVAD: a thrombus may occlude it completely, and even a small clot within or at the tip of the catheter can cause the TIVAD to function poorly (it becomes ''stiff''), and it can also act as a nidus for infection. It was suggested by some that these patients should take long-term aspirin prophylaxis,[2,15] but this is not something we wish to do in children, given the defined albeit small risk of Reye syndrome. Older patients in whom a defect is discovered should also be counseled about the use of oral contraceptives (there is a 35-fold increased risk of thrombosis if a woman with factor V Leiden mutation takes them[33]) and future surgery.[20] The issue about short-term prophlyaxis for surgery is not straightforward, and there is no clear consensus, although if there is a history of venous thromboembolism, it is recommended.[12] Since many of the defects are inherited, it may be appropriate to counsel and test other asymptomatic family members.[12,20] However, there is no evidence that prophylaxis should be given to asymptomatic but affected members of thromboembolism-prone families.[12,13,32] It is critical that a hematologist be involved in all these decisions.

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