The role of coagulation abnormalities in the development of Perthes' disease

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Recent reports have suggested an association between Perthes' disease and an underlying thrombophilic or hypofibrinolytic tendency. In Northern Ireland there is a high incidence of Perthes' disease (11.7 per 100 000 or 1 in 607 children) in a stable paediatric population.

We reviewed 139 children with Perthes' disease and compared them with a control group of 220 aged- and gender-matched healthy primary schoolchildren with similar racial and ethnic backgrounds.

There were no significant deficiencies of antithrombotic factors protein C, protein S, antithrombin III or resistance to activated protein C. A total of 53 (38.1%) of the children with Perthes' disease had a prolonged activated partial thromboplastin time (> 38) compared with 13 (5.9%) of the control group ($p < 0.001$). Our findings have shown that using standard assays, thrombophilia secondary to antithrombotic factor deficiency or resistance to activated protein does not appear to be an aetiological factor for Perthes' disease. The cause of the prolonged activated partial thromboplastin time, usually associated with a clotting factor deficiency, is under further investigation.

J Bone Joint Surg [Br] 2000;82-B:744-6.

Received 10 May 1999; Accepted after revision 14 October 1999

Much has been written about Perthes' disease, yet little is understood regarding its aetiology. Recently, there has been increased interest in the possible role of the coagulation system. The condition arises after ischaemic infarction,

venous or arterial, of the capital femoral epiphysis as was demonstrated by Salter and Thompson¹ and by others^{2,3} in experiments in animal models. Radiologically, avascular necrosis has been demonstrated both on bone scanning⁴ and MRI.⁵

Given this clinical and radiological picture, it is attractive to seek an underlying coagulation defect to explain the infarction. Several studies have suggested an association between antithrombotic factor deficiencies, hypofibrinolysis and the subsequent development of Perthes' disease.⁶⁻⁹

Conditions associated with an increased tendency for thrombosis are considered to be thrombophilic or hypercoagulable states. The former can arise from inherited deficiencies of the antithrombotic factors protein C (PC), protein S (PS) and antithrombin III (ATIII). PC is activated by a thrombin/thrombomodulin complex at the endothelial cell surface. Once activated it binds with its co-factor, PS, to neutralise Factors Va and VIIIa in the clotting mechanism, thus preventing further production of thrombin. Children have lower circulating levels of PC than adults¹⁰ and lack of these factors has been associated with osteonecrosis in adults.^{11,12} Resistance to activated protein C (APC-R) is an inherited thrombophilic trait usually associated with a CGA-CAA substitution at position 1691 on the *Factor V* gene, referred to as *Factor V Leiden*. This is the most common form of heritable thrombophilia.¹³ Since the publication of the original articles by Glueck et al,^{11,12} other investigators have failed to reproduce their findings.^{14,15} Previous studies have had certain limitations; all have involved small numbers of children and none has had clearly defined age- or gender-matched control groups. In most cases the control group has consisted of children admitted to hospital for a variety of day-case procedures.

Northern Ireland has a stable paediatric population. A centralised orthopaedic service serves the whole province. From data generated by the regional information technology unit we found that 313 children had been diagnosed with Perthes' disease during a seven-year period, giving an annual incidence of 11.7 per 100 000 children with a cumulative risk of 1 in 607. Our aim was to review these children and to assess their thrombophilic status compared with an age- and gender-matched control group of healthy primary schoolchildren.

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0301-620X/00/510183 \$2.00

Patients and Methods

Study group. We reviewed 139 children with Perthes' disease at weekly clinics. Blood samples were obtained from all 115 boys and 24 girls. Their mean age was 5.6 ± 1.95 years at the onset of the condition and 8.75 ± 2.01 years at the time of the study. Eight cases were bilateral. A social history was taken and a physical examination carried out. Enquiries about concurrent illnesses and drug therapy were made as these could interfere with the coagulation status. Ethical approval for the study was granted by the Research Ethics Committee of the Queen's University of Belfast.

Control group. A total of 220 healthy primary school-children acted as a control group. The mean age of the 128 boys and 92 girls was 8.85 ± 1.7 years. They were selected at random from schools throughout the province. Full written, informed, consent was gained from the parents beforehand and verbal consent was given by the child before a blood sample was obtained.

Analysis of clotting factors. All blood samples were collected from an antecubital vein, between 09.30 and 12.00 hours, into 3.2% (i.e., 0.109 M) trisodium citrate anticoagulant in a 9:1 ratio of blood to citrate. They were stored at 4°C and transported to the laboratory at the end of the clinic. Immediately upon arrival they were centrifuged for ten minutes at 10 g and analysed for prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin clotting time (TCT), fibrinogen (FIB), PC, PS, ATIII and APC-R. The plasma was then passed through a 20 µm filter and stored at -70°C for further analysis.

All the tests were undertaken using Stago Diagnostica (Parsippany, New Jersey) STA analysers and Stago reagents unless otherwise stated. PT was obtained from a clotting-time assay (STA-Neoplastine C1 Plus). The normal accepted range for children is 12 to 17 seconds. APTT was obtained by a standard clot detection assay using ACTIN FS (DADE) with incubation for four minutes. The normal range is 24 to 38 seconds. ATIII and PC levels were obtained by chromogenic assay (STA-STACHROM ATIII and STA-STACHROM Protein C kits). PS was determined using a clotting-time assay (STA-STACLOT Protein S kit). APC-R was analysed using a modified APTT-based assay, the test plasma being diluted in Factor-V-deficient plasma both in the presence and absence of PC (APC Resistance kit; Chromogenix, Mölndal, Sweden).

Statistical analysis. The results were not normally distributed and therefore we used the Mann-Whitney U test for non-parametric populations to compare the statistical significance. The median values for the blood results of the study group were compared with those of the control group.

Results

Study group. The 139 children from whom a blood sample could be obtained had physical and radiological findings in

keeping with a diagnosis of Perthes' disease. They were otherwise well and none was receiving corticosteroids. When compared with those who were not enlisted in the study there was no significant difference in age at onset, gender or bilaterality. It was therefore concluded that this was a representative study group.

Table I outlines the median values for the results and the interquartile ranges for the different factors.

Analysis of clotting factors. The PT reflects the procoagulant activity of Factors II, V, VII and X. No significant difference in the median values for the PT (12.8 v 12.75s), TCT (17.6 v 17.7s) or FIB (3.01 v 3.01s) was demonstrated.

Activity levels of PC above 60% are considered normal. Two children from the study group and three from the control group had levels below 60%. When we compared the median value in the two groups (83 v 77) the study group had a higher level of PC than the control group. Similar findings were present for PS (101 v 92.5). Indeed the control group contained the only true case of low PS at 27%. ATIII inactivates thrombin and other serine proteases. It therefore acts as a physiological anticoagulant. There were no cases of reduced ATIII in either group. APC-R has recently been described as the most common inherited thrombophilic trait. Eight of the 139 children with Perthes' disease were APC-R positive (5.8%) as were six of the control group (2.7%). No ethical approval had been obtained for genetic testing. Therefore DNA testing for the *Factor V Leiden* mutation was not undertaken in either group.

The APTT provides an assessment of the function of Factors VIII, IX, X, XI and XII. In the study group 53 of the children (38.1%), 46 boys and seven girls, had times greater than 38 seconds (normal range, 24 to 38) in contrast with 13 of the control group (5.9%). The median APTT for the study group was 37.1 whereas that for the control group was 33.8 ($p < 0.001$). Repeat analysis on a random sample of cases with a prolonged APTT revealed consistently high values. In our study all cases with prolonged APTT were diluted in a 50:50 mix with normal plasma. The APTT remained prolonged suggesting the presence of an inhibitor rather than a factor deficiency.

Table I. A comparison of the median values and interquartile ranges for the clotting parameters measured in the study group of 139 children with Perthes' disease and 220 control children

Variables	Study group	Control group
PT (s)	12.8 (11.3 to 13.4)	12.75 (12.3 to 13.1)
APTT (s)	37.1 (34.6 to 38.5)	33.8 (32.2 to 35.3)
TCT (s)	17.6 (17 to 18.1)	17.7 (17 to 18.3)
FIB (g/l)	3.01 (2.62 to 3.42)	3.01 (2.69 to 3.33)
PS (%)	101 (84 to 114)	92.5 (79 to 104)
PC (%)	83 (74 to 91)	77 (71 to 88)
ATIII (%)	114 (108 to 120)	117 (110 to 122)

Discussion

When considering aetiological factors in the development of Perthes' disease, most attention has so far been given to deficiencies in the antithrombotic factors, APC-R and hypofibrinolysis as represented by reduced levels of stimulated tissue plasminogen activator or elevated levels of plasminogen activator inhibitor.

Glueck et al.⁸ initially reported that 23 out of 44 children had factor deficiencies and subsequently found 23 out of 64 children with APC-R.⁹ These studies did not comment on the APTT. Our findings did not reveal significant factor deficiencies or levels of APC-R (5.8%) and are in keeping with more recent studies.^{16,17} The only report which has commented previously on the APTT was that by Hayek et al.,¹⁶ who demonstrated no abnormalities. The APTT was part of the basic coagulation profile carried out in this study. Glueck et al. used a standard APC resistance assay which is a poor predictor for *Factor V Leiden* and is influenced by an abnormal APTT.¹⁸ This may explain the high proportion of patients reported to have APC-R. The modified assay using Factor-V-deficient plasma for APC-R is so sensitive for defects in the Factor V molecule, principally *Factor V Leiden*, that APC-R patients are likely to be *Leiden*-positive. The remaining studies are limited by small numbers and poorly defined control groups and the results should be interpreted with caution.

The ratio of boys to girls in this sample is approximately 5:1. This would be expected in a population of children with Perthes' disease. Inherited thrombophilia is not gender-linked and affects both men and women equally. It can therefore not explain, in isolation, the increased prevalence of the disease in boys.

An interesting, although unexpected finding was a prolonged APTT in a large number of children with the condition. A significant difference was demonstrated when the median APTT value for the study group was compared with that of the control group. Although the APTT provides an assessment of the function of Factors VIII, IX, X, XI and XII, it is unaffected by the levels of PC or PS.

The APTT can be prolonged in several clinical situations, usually related to deficiencies of clotting factors. All, except Factor-XII deficiency, have an increased bleeding tendency and the APTT is used to monitor the anticoagulant effect of intravenous heparin. A circulating anticoagulant can also prolong the APTT and commonly takes the form of an antiphospholipid antibody. These include lupus anticoagulant and anticardiolipin antibody. They exert their anticoagulant effect in vitro; however, in vivo, they predispose to arterial and venous thrombosis.¹⁹ In our study, the APTT remained prolonged after dilution in a 50:50 mix with normal plasma suggesting the presence of an inhibitor rather than a factor deficiency.

A sample group of eight patients with Perthes' disease with prolonged APTT was tested for antiphospholipid antibodies, factor deficiencies including von-Willebrand factor,

fibrinolytic parameters and homocysteine. As yet no abnormalities have been detected.

Further studies are in progress to investigate levels of Factor XII, pre-kallikrein and high-molecular-weight kininogen, factors known to be associated, albeit rarely, with a predisposition to hypercoagulability and also with prolongation of the APTT test. The presence of antiphospholipid antibodies in children is often a transient postinfective phenomenon. It is not clear from the current literature to what extent and with what frequency the transient, or even persistent, antibodies contribute towards thrombotic events in children.

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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