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# The Role of Protein C Deficiency in the Etiology of Perthes Disease

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## SUMMARY

**Background.** Recent reports support the hypothesis that thrombophilia plays an important role in the pathogenesis of Perthes disease (PD) and the objective of this report is to show evidence of the role of protein C deficiency in the etiology of PD, based on a meta-analysis of current scientific literature.

**Material and methods.** Studies were selected in all languages over the last twenty years (1986 to 2006) in MEDLINE, LILACS and EMBASE data bases. The inclusion criteria involved controlled studies, those that presented protein C as a continuous variable, and studies conducted in children with Perthes disease. The fixed effect model for continuous data was used; differences between groups were assessed by the t-test, Z-test and Cochran Q test for independent data and the level of significance was $p < 0.05$.

**Results.** The selected studies involved 175 patients and 193 control subjects. The selected studies were shown to be heterogeneous, but there were no statistically significant differences in protein C levels between groups.

**Conclusion.** The authors' findings were unable to support the hypothesis that protein C deficiency is associated with Perthes disease and that it may play an important role in the ethiopathogenesis of avascular necrosis of the femoral head in childhood.

**Key words:** Perthes, thrombophilia, bone, necrosis

## BACKGROUND

Legg-Calvé-Perthes disease is a common pediatric hip disorder involving the femoral capital epiphysis [1]. Histopathologic changes support the belief that repeated vascular interruption of the blood supply results in venous occlusion with subsequent venous hypertension and bone avascular necrosis [2]. This suggests that thrombotic events may be one of the underlying causes of Perthes disease (PD), and recent reports support the hypothesis that thrombophilia plays an important role in the pathogenesis of PD [3,4[.

Thrombophilia or hypofibrinolysis may predispose to thrombotic venous occlusion that leads to bone necrosis. Protein C (PC), protein S (PS) and antithrombin III (ATIII) are three naturally occurring physiologic anticoagulants that are part of the negative feedback system of blood clotting. Inherited abnormalities of these compounds (resistance to activated protein C, protein C deficiency and protein S deficiency) cause thrombophilia and a tendency to clot [2,3,4,5]. Activated protein C resistance is the commonest cause of inherited thrombophilia and several studies have found a high prevalence of abnormal levels of PC in patients with PD [3,4].

The objective of this report is to show evidence of the role of protein C deficiency in the etiology of Perthes disease, based on a meta-analysis of current scientific literature.

## MATERIAL AND METHODS

### Search for relevant studies

The search was conducted over the last twenty years (1986 to 2006) in MEDLINE, LILACS and EMBASE data bases, for clinical trials that evaluated the relationship between Perthes disease and thrombophilia. Studies were selected in all languages and the following terms were used for the search, either alone, or in combination: Protein C; Perthes disease; fibrinolysis; thrombophilia; avascular necrosis; femoral head; children; coagulation. The bibliographies and reference lists of retrieved articles were also scanned to supplement the electronic search.

### Study selection and data abstraction

The inclusion criteria involved controlled studies, those that presented protein C as a continuous variable, and studies conducted in children with Perthes disease. Sample size, time of onset of Perthes disease and laboratory assay were not exclusion criteria. The studies were classified according to the levels of evidence suggested by the Center of Evidence-Based Medicine [6] (Table 1). From each selected article, the following baseline information was abstracted: source of data, study design, inclusion and exclusion criteria, and demographic data of study participants, including sample size, age and sex.

### Statistical methods

All studies selected were presented in descriptive statistics and distributed in tables. The absolute mean differences in the concentration of protein C between the intervention and control groups were converted into standardized mean differences; the fixed effect model for continuous data was used and the heterogeneity of results across the studies tested, using a Cochran Q test [7]. If significant heterogeneity was present ($p < 0.05$), then a random effect meta-analysis model was used [7]. The results of the meta-analysis are presented in tables and a chart.

Results were reported as effect size of means and their confidence intervals. Differences between groups were assessed by the t-test, Z-test and Cochran Q test for independent data and the level of significance was $p < 0.05$.

## RESULTS

The original search yielded 43 reports, but only five of them completely met the inclusion criteria and were selected for the meta-analysis. The LILACS and EMBASE databases did not contribute to the search results. The selected studies were the articles by Metha et al 82006, Yilmaz et al 9 2005, Pózán et al 10 2003, Koo et al 11 2002 and Sirvent et al 12 2000. These studies involved 175 patients and 193 control subjects. Male-to-female ratios were not available in three studies (Metha et al 8 2006, Pózán et al 10 2003, Sirvent et al12 2000); in the Yilmaz et al9 2005 and Koo et al 200211 studies, there were 113 males (59 patients and 84 controls) and 24 females (13 patients and 21 controls).

It was not possible to analyze the results according to age, sex and severity of the disease. In spite of

Tab. 1. Features of the selected studies

| Study | N of control group | N of patient group | Level of quality |
|---|---|---|---|
| Metha et al 2006 | 36 | 51 | III |
| Yilmaz et al 2005 | 79 | 46 | III |
| Pózán et al 2003 | 30 | 30 | III |
| Koo et al 2002 | 26 | 26 | III |
| Sirvent et al 2000 | 22 | 22 | III |

differences in laboratory tests, results were presented in the same scale and were thus equivalent. The results are shown in Tables 1 and 2, and Figure 1.

## DISCUSSION

The etiology of PD is still uncertain and belongs to the "idiopatic" group. Intravascular thrombosis (IT) seems to form the main mechanism in the pathogenesis of the disease [2]. This suggests that thrombophilia plays an important role in the pathogenesis of PD [3,4]. Low protein C levels are a common cause of thrombophilia and may thus represent an important associated risk factor for inherited thrombophilia. Several reports have found a possible etiologic association between PD and protein C deficiency [2,3,4].

Glueck et al 3 1994 found a high prevalence of low levels of PC (nineteen out of forty-four) and protein S (four out of forty-four) in PD patients and supported the hypothesis that inherited thrombophilia was the underlying cause of the disease. Other studies offered no support for a causative relationship between inherited thrombophilia and PD [5,13,14]. Studies on this subject mostly involved small patient populations or were not controlled, and their results lacked the statistical power to refute or accept the association between IT and PD.

In accordance with the inclusion criteria, only five reports were selected for the meta-analysis. These were the articles by Metha et al 82006, Yilmaz et al 9 2005, Pózán et al 10 2003, Koo et al 11 2002 and Sirvent et al 12 2000. The selected studies were shown to be heterogeneous (by Cochran Q test); nevertheless, no classical sensitivity analysis was done, because of the small number of the studies selected. However, it was found that the major source of heterogeneity was small sample size and lack of power (Table 2 and Figure 1).

The studies have some methodological limitations, such as small sample size in the cases of Koo et al 11, Sirvent et al 12 and Pózán et al 10. In the Pózan et al 10 study, there was an inappropriate control group that included some adults; since it is known

Tab. 2. Random effect model meta-analysis of the selected studies

| Study | Effect size | Confidence interval |
|---|---|---|
| Metha et al 2006 | 0.853 | 0.405 to 1.302 |
| Yilmaz et al 2005 | 0.501 | 0.129 to 0.876 |
| Pózán et al 2003 | -0.353 | -0.848 to 0.142 |
| Koo et al 2002 | -0.843 | -1.357 to -0.329 |
| Sirvent et al 2000 | -0.027 | -0.565 to 0.619 |
| Combined studies | 0.143 | -0.065 to 0.353 |

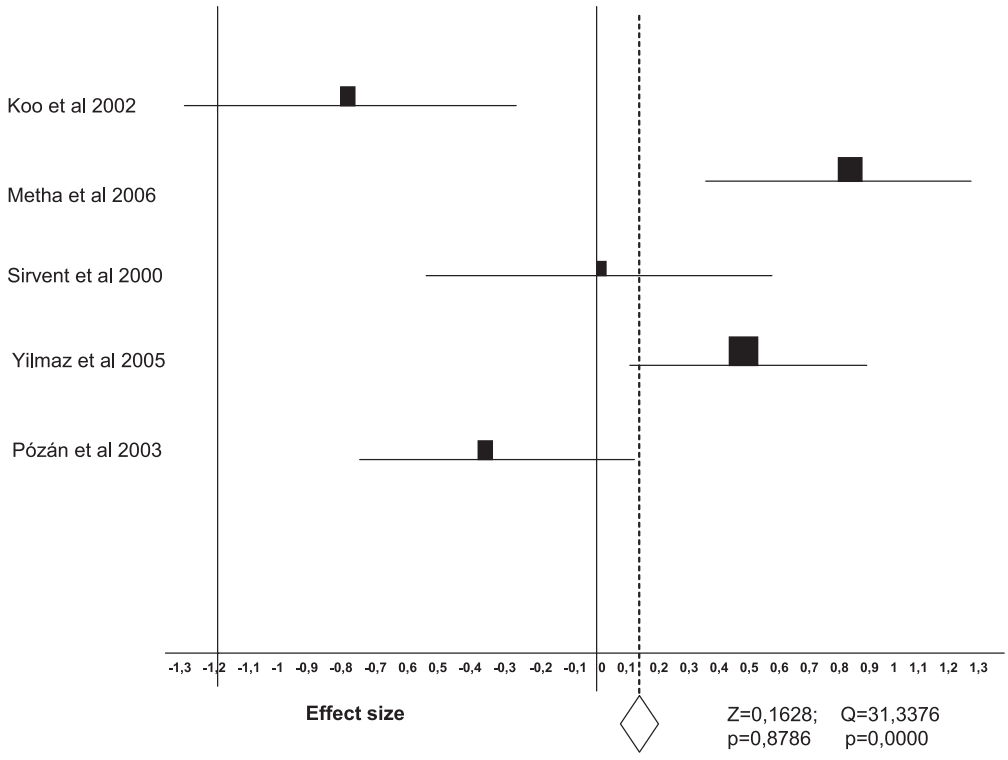


Fig. 1. Meta-analysis of protein C deficiency in Perthes disease

that protein C levels are age-dependent, it would be incorrect to do this. Finally, the meta-analysis consisted of reports of Level III evidence (case-control) and studies of this type are not designed to prove etiology, but only to find association between variables.

All the studies were considered heterogeneous and for this reason, a random effect model analysis was performed. In total there were 175 patients and 193 control subjects. A meta-analysis corrects some of the problems of individual studies, and improves the confidence and evidence levels of the results; in this instance, it gave more accuracy and precision to the combined results of the individual studies, based on an adequate pooled sample size and power. In this meta-analysis, only continuous values were used, in order to prevent bias, because some reports that presented only normal and abnormal values did not find any abnormal individuals in at least one of either control or case groups, or both.

The authors' findings were unable to support the hypothesis that protein C deficiency is associated with Perthes disease and that it may play an important role in the ethiopathogenesis of avascular necrosis of the femoral head in childhood. These data suggest that PC might not completely explain the role of thrombophilia in the etiology of PD. However, PC deficiency itself is not enough to exclude thrombophilia as an important cause of PD or NAV.

A tendency for early thrombosis may be caused by various disorders. Deficiencies of natural anticoagulants (such as ATII, PS), heritable resistance to APC caused by factor V Leiden mutation, elevated plasma homocysteine and high levels of antiphospholipid antibodies (anticardiolipin and lupus anticoagulant) are important risk factors for occlusive arterial disease and venous thromboembolism [2,3,4,5]. Arruda et al 15 suggested that factor V mutation was the only inherited risk factor associated with the development of PD. Balasa et al 16 found that PD was associated with familial thrombophilia (factor V Leiden mutation) and acquired thrombophilia (anticardiolipin antibodies). In contrast to these positive associations, the Lopez-Franco et al14 study did not support the association between factor V Leiden mutation and PD.

The fact that inherited thrombophilia was found in a large proportion of PD patients suggests that inherited or acquired venous thrombosis may be an important risk factor in the ethiopathogenesis of avascular necrosis of the femoral head. Several studies have evaluated the family history of risk factors for inherited thrombophilia or venous thrombosis. Glueck et al4 reported the presence of a history suggesting inherited thrombophilia in 58% of relatives of PD patients.

The role of other causes of thrombophilia differing from PC deficiency was not analyzed, and the idea that PD may be associated with resistance to activated protein C (factor V Leiden mutation), acquired thrombophilia and so on, could not be completely refuted. Nevertheless, the authors believe that PD must now be understood as a syndrome caused by multiple disorders and not as a specific "idiopathic" disease. Thus, inherited or acquired thrombophilia (like sickle cell anemia or corticoid use) may be one of the etiologic causes of this syndrome, characterized by avascular necrosis of the femoral head in childhood.

Further studies should focus on proving the role of PS, ATII, APC, acquired thrombophilia and factor V Leiden mutation in the pathogenesis of PD. The true role of protein C in the etiology of Perthes disease seems to have been explained, but the authors suggest large controlled studies with adequate power and sample size to confront the results of the present meta-analysis.

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