Non-traumatic necrosis of bone (osteonecrosis) is associated with endothelial cell activation but not thrombophilia

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Objective. The pathophysiology of non-traumatic osteonecrosis (ON) or avascular necrosis (AVN) of the femoral head remains poorly understood. Some studies have suggested the contribution of underlying thrombophilia as a mechanism; however, no specific thrombophilic factor has been consistently found in association with the disease. We are presenting data suggesting a role for endothelial cell activation rather than thrombophilia in ON.

Methods. A prospective consecutive cohort of 49 patients with a diagnosis of ON. The disease was considered idiopathic in five and secondary in 44 patients. The investigation included a coagulation and thrombophilia profile, endothelial cell activation and non-specific inflammatory markers as well as a biochemical profile. Statistical analysis using Fisher’s exact test was obtained to assess correlation between endothelial cell markers and variables of inflammation.

Results. Patients with non-traumatic ON were not found to have a specific underlying thrombophilic factor compared with the general population. Out of 49 patients, 19 had elevation of at least one endothelial cell markers. We found that activation of endothelial cell markers was independently correlated to ON but not correlated to the presence of inflammation (P = 1.0000).

Conclusion. These results suggest that non-traumatic ON is not associated with a specific thrombophilic abnormality in those affected. This study demonstrates a potential association between regional endothelial dysfunction and ON. More studies are needed at a molecular level to further investigate the specific role of endothelium in the physiopathology of ON. A better understanding of the underlying mechanism could lead to potential preventive and therapeutic strategies of this devastating disease.

KEY WORDS: Avascular necrosis, Osteonecrosis, Thrombophilia, Endothelial cell activation, Inflammation.

Introduction

Osteonecrosis (ON) or avascular necrosis (AVN) of the femoral head is a clinical entity in which bone death occurs as a result of interruption of blood at the level of microcirculation. The aetiology and pathogenesis of non-traumatic AVN have not been fully elucidated. The understanding of this disease progression is important for the following reasons: (i) AVN is a devastating musculoskeletal condition that strongly impacts on those affected; (ii) it tends to occur in young people; (iii) current treatments are sub-optimal since they are for the most part palliative rather than curative; and (iv) we are currently unable to identify individuals who will develop AVN in those considered at high risk and as such, practitioners are not able to proactively treat the patient or stop the progression of the disease once AVN has developed.

Although the actual prevalence of the disease is unknown, an estimated 10,000 to 20,000 new patients with AVN are diagnosed each year in the United States [1]. While there is no sex predilection confirmed yet, it appears that the male population might be more frequently affected, based on reported studies. AVN is the underlying diagnosis in 5–18% of the more than 500,000 total hip arthroplasties performed yearly in the United States and Western Europe [1].

AVN has been associated with a variety of risk factors and classified as either secondary or idiopathic. Environmental risk factors include use of corticosteroids, hyperlipidaemia, use of alcohol, various blood dyscrasias (haemoglobinopathies), pregnancy, hyperbaric exposure, use of chemotherapeutic agents, SLE, inflammatory bowel disease (IBD), lipid storage diseases and familial thrombophilias [1]. There is also a high incidence of AVN among organ transplant recipients. Allogenic bone marrow transplantation carries the highest incidence of AVN, reported to be 10% in the United States, with an increased incidence of AVN in the patient receiving allograft marrow [2]. Some studies have described AVN in association with HIV-infected patients [3], and after septic shock, multiorgan failure and renal transplantation [4].

Several early studies have also implied that alcohol and steroid use may account for 90–100% of all non-traumatic ‘secondary’ cases [5]. The effects of both alcohol and corticosteroids have been related to alterations in lipid metabolism through intravascular coagulation and fat embolism phenomena. However, of those people fulfilling risk criteria for AVN development, most never develop the disease. This finding suggests that a second event appears to be necessary to initiate the development of AVN in a subset of patients with an acquired or inherited predisposition for this disorder [5].

Inherited and/or acquired thrombophilia (proteins C and S deficiency, factor V Leiden, Prothrombin 20210A mutation, aCLs) and hypofibrinolysis (low tPA level) or high levels of lipoprotein(a) have been associated with AVN of the femoral head and/or the mandible in adults and with AVN (Legg–Calve–Perthes disease) in children [6]. High plasminogen activator inhibitor-1 (PAI-1) levels has also been associated with AVN of the femoral head [7–9]. However, other authors were unable to confirm these results and thrombophilia-associated AVN remains controversial. A more recent study reported an association between von Willebrand factor (vWF) and AVN [10].

The present pilot study was conducted to evaluate the contribution of a specific thrombophilic factor and/or endothelial
cell activation in the development of non-traumatic ON, and to further assess if endothelial cell activation is independent of the presence of inflammation.

Materials and methods

The study was given ethical approval by the institutional review board at two different institutions and signed informed consent was obtained from all patients. A total initial cohort of 54 patients was recruited for the study. Upon detailed review of their history and hospital chart, five patients were excluded on the basis of not fulfilling the inclusion criteria. Thus, a final number of 49 patients with a diagnosis of non-traumatic ON of the femoral head were included. The study was designed as a preliminary (pilot) study and as such, did not include healthy control subjects.

The 49 patients were referred to the Department of Orthopaedic Surgery at the Montreal General Hospital, McGill University Health Centre for further evaluation and treatment. ON was documented by history, physical evaluation and radiological evaluation. All patients had a plain radiograph as the first radiological diagnostic test, which was followed for all patients by MRI. The diagnosis of ON and stage of disease were established by an experienced orthopaedic surgeon and bone radiologist, based on specific criteria such as subchondral collapse and flattening of the femoral head. The extent of the necrotic lesion was assessed by MRI. Characteristic patterns of ON constituted typical signal alteration of the necrotic bone surrounded by a band of low signal intensity on T1-weighted images, representing the new bone formation in the reactive interface, as previously described [11].

Blood analysis

All patients of the cohort were evaluated for the following indices: CBC (complete blood count), creatinine, electrolytes, liver function tests, international normalized ratio (INR), activated partial thromboplastin time (aPTT), protein C, protein S, anti-thrombin III, PAI-1 antigen, plasminogen, activated protein C resistance (APCr), Factor VIII, vWF, erythrocyte sedimentation rate (ESR), fibrinogen, CRP [human serum CRP (hsCRP)], lupus anti-coagulant, aCLs and genetic analysis [methylene tetrahydrofolate reductase (MTHFR), factor V Leiden, PAI-1 genotypes statistical analysis]. All data was collected in a single database that was approved by the institutional review boards. A final analysis using SPSS software (SPSS Chicago) was carried out. Data analysis had an assigned significance level of 0.05. Fisher’s exact test was obtained to assess correlation. SAS/genetics version 9.1.3 was used for PAI-1 genotypes statistical analysis.

Results

All data was collected in a single database that was approved by the institutional review boards. A final analysis using SPSS software (SPSS Chicago) was carried out. Data analysis had an assigned significance level of 0.05. Fisher’s exact test was obtained to assess correlation. SAS/genetics version 9.1.3 was used for PAI-1 genotypes statistical analysis.
in 3/49 (6%) patients and solid-organ transplant in 2/49 (4%) patients (Fig. 1). Most patients (35/49 or 71%) had bilateral hip involvement and most had advanced disease (Stages IV–VI) [12]. Most patients (44/49 or 90%) did not have a personal history of thrombosis. Out of five patients who developed a thrombosis, two patients had a thrombotic event which occurred in a post-operative context. In these two patients, thrombophilia was also found: one patient was positive for homozygous MTHFR mutation and the other with a combined mild deficiency of protein C (0.64) and protein S (0.56) based on one single measurement (suggesting a iatrogenic cause accounting for the deficiencies). One patient had an MI (myocardial infarction) at 28 yrs of age but with negative thrombophilia; this same patient was also known for SLE, pseudotumour cerebri, hypercholesterolaemia and hypertension. One other patient with personal history of DVT but negative thrombophilia profile was known with dermatomyositis, Raynaud’s disease and hypercholesterolaemia. The last patient with a past thrombotic event [pulmonary embolism (PE)] also had a negative thrombophilia profile and was known for lymphoma, Blackfan–Diamond anaemia and secondary iron overload. Risk factors for thrombosis (venous/arterial) such as CTD, cancer, hypercholesterolaemia and hypertension were thus present in these last three patients who had a thrombotic event in the absence of underlying thrombophilia.

There was no association found between a specific thrombophilic marker and AVN in this cohort of non-traumatic AVN patients. The results obtained in our AVN cohort were comparable with published results obtained in a healthy control population (composed of 474 control subjects) defined with the following criteria in the Leiden Thrombophilia Study (LETS): no history of venous thromboembolism, no use of coumarin following criteria in the Leiden Thrombophilia Study (LETS): no history of venous thromboembolism, no use of coumarin or anti-platelet therapy, and no family history of thrombophilia. The deficiencies reported in our AVN cohort for hypercoagulability/thrombophilic markers were based on a sole measurement. A decrease in plasminogen level (<0.8 UI) was observed in 2/49 (4%) patients. Lupus anti-coagulant was negative in all patients. IgM aCLs were positive in 1/49 (2%) patients and the results were based on one measurement: 6/7 MPL titres were between 23–30 and 1/7 was 41.9 MPL. Underlying diagnosis in these patients were the following: post kidney transplants (2/7), Hodgkin’s lymphoma (1/7), MCTD (2/7) and oligoastrocytoma (1/7). There was no specific comorbidity in 1/7 patient with positive IgM isotype. IgG aCLs were positive in 1/49 (2%) patient (15.8 GPL) (Table 1). Prothrombin 20210A as well as factor V Leiden heterozygous mutations were also negative in all patients, whereas homozygous MTHFR was found in a proportion comparable with healthy controls. PAI-1 antigen level was normal in all patients. There was no allelic or genotypic association or trend found for each PAI-1 genotype analysed (4G/4G: P = 1.000, HindIII: P = 0.158, CA repeat: P = 0.662) (Table 2).

From the results on the endothelial cell markers, out of 49 patients, 11 (22%) had increased vWF antigen levels, 6 (12%) had increased factor VIII levels and 18 (37%) had increased vWF activation using at least two abnormal endothelial cell markers. PAI-1 antigen level was normal in all patients. There was no allelic or genotypic association or trend found for each PAI-1 genotype analysed (4G/4G: P = 1.000, HindIII: P = 0.158, CA repeat: P = 0.662) (Table 2).

CoRistocetin levels (Table 3). In 4/11 (36%) patients, the vWF antigen level was >2.50 U/ml and 4/18 (22%) patients had increased CoRistocetin level >2.0 U/ml. The diagnosis of endothelial cell activation using at least two abnormal endothelial cell markers was found in 25% of patients (12/49) and one abnormal endothelial cell marker was seen in 39% (19/49) patients (Table 4). Inflammatory markers showed the following: hsCRP (CRP) was increased in 9/49 (18%) of patients, ESR was elevated in 36/49 (78%) patients and Fibrinogen level was increased in 2/49 (4%) patients. All patients (except one) with elevated hsCRP and fibrinogen level had an elevated ESR. For this reason, ESR was the sole inflammatory criteria used for analysis. To ascertain that the increase in endothelial cell markers observed was not a result of inflammation (acute-phase reactant), we correlated elevated ESR results with abnormal values of endothelial cell markers using Fisher’s exact test and showed no correlation when each variable is evaluated alone (P ≥ 0.41) or when two variables are put together (P ≥ 1.00) suggesting that endothelial cell activation is, at least in part, implicated in the up-regulation of these markers (Table 5).

### Table 1. Hypercoagulability/thrombophilic markers

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of AVN Patients (Total n = 49) (%)</th>
<th>Expected in healthy controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-thrombin III deficiency</td>
<td>1 (2)</td>
<td>0.2–1.9 [13]</td>
</tr>
<tr>
<td>Protein C (activity) deficiency</td>
<td>3 (6)</td>
<td>1.0 [13] [0.5–4.0 [36–38]</td>
</tr>
<tr>
<td>Protein C (antigen) deficiency</td>
<td>0 (0)</td>
<td>0.4–1.5 [13] [0.5–4.0 [36–38]</td>
</tr>
<tr>
<td>Total Protein S deficiency</td>
<td>1 (2)</td>
<td>1.3–2.3 [13]</td>
</tr>
<tr>
<td>IgG aCLs (pos &gt; 15U GPL/ml)</td>
<td>1 (2)</td>
<td>6.5 [22]</td>
</tr>
<tr>
<td>IgM aCLs (pos &gt; 23U MPL/ml)</td>
<td>7 (14)</td>
<td>9.4 [22]</td>
</tr>
<tr>
<td>Lupus anti-coagulant</td>
<td>0 (0)</td>
<td>2–4 [39]</td>
</tr>
</tbody>
</table>

### Table 2. Thrombophilic markers/genetic mutation analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of AVN Patients (Total n = 49) (%)</th>
<th>Expected in healthy controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR (homozygous)</td>
<td>7 (14)</td>
<td>1.5–15 [40]</td>
</tr>
<tr>
<td>Factor vs Leiden (heterozygous)</td>
<td>0 (0)</td>
<td>1–5 [13]</td>
</tr>
<tr>
<td>PAI-1 4G/4G genotype</td>
<td>Absence of allelic, genotypic association or trend</td>
<td>P = 1.000</td>
</tr>
<tr>
<td>PAI-1 HindIII genotype</td>
<td>Absence of allelic, genotypic association or trend</td>
<td>P = 0.158</td>
</tr>
<tr>
<td>PAI-1 STR genotype</td>
<td>Absence of allelic, genotypic association or trend</td>
<td>P = 0.662</td>
</tr>
<tr>
<td>PAI-1 antigen (&gt;115 ng/ml)</td>
<td>0 (0)</td>
<td>Variable*</td>
</tr>
</tbody>
</table>

*Vary according to PAI-1 genotype.

### Table 3. Endothelial cell activation markers

<table>
<thead>
<tr>
<th>VWF : Ag</th>
<th>VWF : CoRistocetin</th>
<th>FVIII</th>
<th>No. of patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>4</td>
<td>4</td>
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<tr>
<td>N</td>
<td>↑</td>
<td>N</td>
<td>7</td>
<td>7</td>
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<tr>
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<td>↑</td>
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<td>N</td>
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<td>6</td>
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<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>30</td>
<td>30</td>
</tr>
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</table>

### Table 4. Detailed analysis of endothelial cell activation markers

<table>
<thead>
<tr>
<th>Variables</th>
<th>Two-sided P</th>
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</thead>
<tbody>
<tr>
<td>VWF-Ag</td>
<td>0.4103</td>
</tr>
<tr>
<td>VWF-CoRistocetin</td>
<td>0.7172</td>
</tr>
<tr>
<td>FVIII</td>
<td>1.0000</td>
</tr>
<tr>
<td>Two abnormal values</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

*Blood group non-O associated with higher levels.

### Table 5. Correlation between inflammation and Endothelial cell activation

CoRistocetin levels (Table 3). In 4/11 (36%) patients, the vWF antigen level was >2.50 U/ml and 4/18 (22%) patients had increased CoRistocetin level >2.0 U/ml. The diagnosis of endothelial cell activation using at least two abnormal endothelial cell markers was found in 25% of patients (12/49) and one abnormal endothelial cell marker was seen in 39% (19/49) patients (Table 4). Inflammatory markers showed the following: hsCRP (CRP) was increased in 9/49 (18%) of patients, ESR was elevated in 36/49 (78%) patients and Fibrinogen level was increased in 2/49 (4%) patients. All patients (except one) with elevated hsCRP and fibrinogen level had an elevated ESR. For this reason, ESR was the sole inflammatory criteria used for analysis. To ascertain that the increase in endothelial cell markers observed was not a result of inflammation (acute-phase reactant), we correlated elevated ESR results with abnormal values of endothelial cell markers using Fisher’s exact test and showed no correlation when each variable is evaluated alone (P ≥ 0.41) or when two variables are put together (P ≥ 1.00) suggesting that endothelial cell activation is, at least in part, implicated in the up-regulation of these markers (Table 5).
Discussion

Thrombophilia and AVN

Over the last decade there has been a considerable interest in better defining the physiopathology of AVN, and many authors have examined a potential underlying aetiological factor, including thrombophilia. A first study of 30 patients, which focused on fibrinolytic activity demonstrated that hypofibrinolysis mediated by high PAI-1 level was prevalent in idiopathic AVN whereas high lipoprotein(a) played an aetiological role in secondary AVN [7, 8]. Glueck et al. [14] reported in a subsequent study of 31 patients that 74% had one or more primary coagulation disorders [resistance to activated protein C, protein C deficiency, high lipoprotein(a) and low tPA level]. Another report revealed in 42 patients that hypofibrinolysis was prevalent (low stimulated tPA level and high PAI-1 level). Also, high titres of aCLs were found in patients compared with controls (22% vs 5% for IgG, P = 0.02) [8]. High levels of aCLs were found by Korompilias et al. [15] who reported similar results in patients with AVN compared with controls (37.5% vs 1%).

Glueck et al. [16] also evaluated 206 consecutive patients to determine the prevalence of abnormal coagulation tests and found evidence of hypercoagulability in 136 of 206 (66%) patients. Resistance to activated protein C and aCLs were the most frequently found thrombophilic defects [16]. Three other well-recognized prothrombotic conditions of importance, the factor V Leiden, the C677T MTHFR polymorphism (homozygous) and prothrombin 20210A gene mutations have recently been evaluated in 59 patients with AVN and no relationship between prothrombin 20210A, MTHFR, factor V Leiden mutations and AVN was found, correlating our findings in the present study [17]. Bjorkman et al. [18] on the other hand found an increased incidence of factor V Leiden or Prothrombin 20210A mutations in 63 patients with non-traumatic ON compared with healthy controls.

Zalavras et al. [19] also found an increased incidence of factor V Leiden in a study population of 72 patients with AVN but not the prothrombin 20210A mutation. Glueck et al. [17] demonstrated that the plasminogen activator inhibitor-1 gene was shifted toward homozygosity for the 4G polymorphism in patients with AVN; 41% of these were homozygous for the 4G/4G polymorphism vs 20% of 40 healthy control subjects.

Intravascular coagulation (or microvascular thrombosis) has been postulated as the common final pathogenetic mechanism for the development of ON [1, 20, 21]. Thrombophilia refers to an increased tendency for thrombosis, and hypofibrinolysis to a reduced ability to lyse thrombi. Both processes can result in microvascular thrombosis and can thus be appealing causes of microvascular thrombosis in ON. These processes are well-known causes as well of arterial and venous thrombotic events as seen in individuals with MI, deep vein thrombosis (DVT) of the lower extremities and pulmonary embolism.

In our cohort of patients with non-traumatic AVN of the femoral head, no specific thrombophilic marker was found to be in association with ON. When each thrombophilic marker in our cohort is compared with the incidence expected in the general population, there is no significant difference seen. We have observed a mild increase in IgM anti-cardiolipin compared with what is reported in healthy individuals, but the significance of IgM anti-cardiolipin in relation with thrombosis is not as clearly established as it is for IgG anti-cardiolipin. Studies indicate that there is a higher prevalence of IgM positives than IgG in the general population with these isotypes occurring in 9.4 and 6.5% of the population, respectively [22]. Many of these antibodies are transient and are not associated with APS [23] or they may occur in association with specific clinical conditions such as haematological and lymphoproliferative malignancies [24]. Some studies report an association between IgM isotype and increased risk of venous thrombosis [25] whereas other studies failed to show a thrombophilic risk [24, 26]. Comparing our results to some of the published data described above, none of the patients in our selected cohort had positive or elevated value for lupus anticoagulant, PAI-1 antigen level or factor V Leiden and prothrombin 20210A mutations. Moreover, no allelic or genotypic association or trend was found for the three different PAI-1 genotypes analysed.

Our findings on the lack of any association between thrombophilia and ON is in itself not surprising. As it is well known, thrombophilia can affect many different vascular beds, venous or arterial. We can easily speculate that individuals with positive thrombophilic markers would therefore not only develop thrombosis at the level of the hip bone but elsewhere as well. Therefore, a concept where known systemic risk factors (thrombophilic markers) for multi-level thrombosis would suddenly manifest locally and specifically to the bone vasculature is unlikely. In our cohort, five patients out of 49 had a thrombotic event (other than the site of ON). The post-operative context (2/5) and the other risk factors present such as cancer, hypercholesterolaemia and inflammatory conditions (CTDs) in the other three patients could easily explain their occurrence. Thrombophilia was found only in association with the two patients with thrombotic events that have occurred post-operatively, suggesting that thrombophilic markers, overall, in our cohort were not major causes for thrombosis.

Endothelial cell activation and AVN

The most interesting finding in our cohort is the elevation in 19 out of 49 patients (39%) of at least one endothelial cell markers, which corroborates Zalavras et al.’s [10] previous finding on the association between vWF and ON. We were able to demonstrate using Fisher’s exact test, that activation of endothelial cell markers were independent (or not correlated) to the presence of inflammation (P = 1.0000). These findings are interesting if we relate to the concept of specific thrombosis inside the bone vasculature. Due to its unique localization, the endothelium is continuously exposed to inflammatory cells and circulating factors, which could induce endothelial activation and/or injury [27]. Endothelial cell activation may thus be seen as one possible explanation for the development of microcirculatory thrombus at the level of microvascular environment of bone, a concept known as regional endothelial dysfunction (RED) [28]. Endothelial cell heterogeneity and vascular-bed-specific haemostasis are well-accepted concepts in the field of vascular biology [29-32]. Furthermore, recent data has demonstrated successful isolation and characterization of human bone-derived endothelial cells from unaffected (control) and affected (AVN) femoral heads from human subjects undergoing surgery for AVN repair, supporting the regional endothelial cell activation hypothesis [33].

In the present clinical pilot study, 39% of patients with AVN had elevation of at least one endothelial cell marker. One possible explanation for the absence of this finding in the whole cohort might be attributed to the different stage of the disease in each patient (some had more advanced disease than others) and the different phase at which the sample was obtained between patients (at time of AVN diagnosis, some may have had the disease for a longer period than others) making it difficult to homogenize the results on these endothelial cell markers. Endothelial cell activation as a potential mechanism for ON is appealing as it converges all multiple known risk factors via a single common pathway. This would unite the wide variety of aetiopathological factors that have been previously associated with the disease into one single explanation.

The discrepancies observed in this study compared with previous published data may be explained on different levels. First, despite the lack of abnormalities detected in a considerable proportion of patients, many published studies concluded on thrombophilia in association with AVN. Second, many studies
have included cases of traumatic AVN and patients with sickle cell disease, where the mechanisms for ON in these subsets of patients may be different than the patients selected in our cohort. Third, some studies concluding on the association between thrombophilia and AVN did not always report on the incidence of other sites of thrombotic manifestations. Fourth, the discrepancy in the results may also be explained by the absence in our study of an age and sex-matched control group, which requires the comparison of our results with the expected prevalence of thrombophilia in a healthy control population. Finally, our results on endothelial cell markers were not adjusted for blood group, although some authors found that the association between vWF and the risk of cardiovascular mortality was independent of blood group [34, 35].

In conclusion, our results suggest that non-traumatic ON is not associated to a specific thrombophilic abnormality in those affected. This study rather demonstrates a potential association between regional endothelial dysfunction and ON. More studies are needed at a molecular level to further appreciate the specific role of endothelium in the physiopathology of this condition. A better understanding of the underlying mechanism could lead to potential preventive and therapeutic strategies of this devastating disease.

Rheumatology key messages
- Non-traumatic ON is a devastating musculoskeletal condition with unclear physiopathology.
- Non-traumatic ON is not associated to a specific thrombophilic abnormality.
- Our study emphasizes on endothelial dysfunction as a model of ON.

Disclosure statement: The authors have declared no conflicts of interest.

References