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Elevations in D-dimer and C-reactive protein are associated with the development of osteonecrosis of the hip in HIV-infected adults

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Abstract

Background—A high incidence of non-traumatic osteonecrosis has been reported in HIV-infected patients. We investigated the levels of D-dimer and C-reactive protein (CRP) in a cohort of HIV-infected adults with and without osteonecrosis of the femoral head.

Methods—43 HIV-infected patients with osteonecrosis of the femoral head and a comparison group of 50 HIV-infected patients with negative MR imaging of the hips and for whom serial plasma samples were available were included. D-dimer and CRP levels were measured prior to and at the time of diagnosis for osteonecrosis patients, at the time of negative MR imaging of the hips for controls, and 6 months later for both groups.

Results—Biomarker levels were elevated at the time of diagnosis in the osteonecrosis cohort compared with controls. Median D-dimer value was 0.32 µg/mL in the osteonecrosis group compared to <0.22 µg/mL in the control group (p=0.016). For CRP the corresponding values were 2.52 mg/L and 1.23 mg/L (p=0.003). Post-diagnosis, D-dimer and CRP levels were also elevated in the osteonecrosis patients compared to controls. Linear regression demonstrated a rise in D-dimer levels from pre-diagnosis to diagnosis in the osteonecrosis patients while CRP levels did not change significantly over time.

Conclusions—Compared to controls, patients who developed osteonecrosis had elevated levels of D-dimer and CRP at diagnosis. D-dimer levels increased while CRP levels did not change

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significantly from pre-diagnosis to diagnosis. These data suggest that patients with higher levels of inflammation are at an increased risk of osteonecrosis.

Keywords

HIV; osteonecrosis; D-dimer; C-reactive protein

Background

A high incidence of non-traumatic osteonecrosis has been reported in HIV-infected patients [1]. Although cases are frequently associated with well-established predisposing factors for osteonecrosis, including corticosteroid use, hyperlipidemia, and alcohol abuse, studies have also identified protease inhibitor use and history of advanced HIV disease as risk factors in this population [2–6]. While the pathogenesis is not well understood, it has been postulated that non-traumatic osteonecrosis develops as a result of intravascular coagulation followed by increased intraosseous venous pressure, reduced arterial flow, and hypoxic bone death. Supporting this, pro-coagulant abnormalities, both inherited and acquired, have been associated with increased risk of osteonecrosis [7, 8]. Our group had previously identified an association between positive anti-cardiolipin antibody and osteonecrosis in HIV-infected adults [6]. No differences were seen in lupus anticoagulant or protein S levels in this cohort compared with HIV-infected controls without osteonecrosis.

In HIV-infected patients, elevated levels of D-dimer, a fibrin degradation product, have recently been associated with increased risk of death and opportunistic infections [9, 10]. Levels of C-reactive protein (CRP), an acute phase protein with both pro-inflammatory and anti-inflammatory effects, are elevated in HIV-infected adults relative to HIV negative controls [11–13]. CRP levels correlate with HIV disease progression independent of CD4+ T-cell count and HIV viral load [14], and predict survival in HIV-infected individuals [13]. In this population, elevated CRP levels have been associated with traditional cardiovascular disease risk factors, including tobacco use and elevated cholesterol, as well as with increased visceral adipose tissue and lipodystrophy [15, 16]. The clinical implications of an elevated CRP and the optimal utilization of CRP levels in the evaluation of HIV infected adults have not yet been established in larger clinical trials, however.

Since coagulation and inflammation are thought to play a role in the development of osteonecrosis, we investigated the levels of D-dimer and CRP in longitudinal samples from a well-characterized cohort of HIV-infected adults with and without osteonecrosis of the femoral head.

Methods

HIV-infected patients with either asymptomatic or symptomatic osteonecrosis of the femoral head enrolled in studies at the NIH Clinical Center and for whom serial plasma samples were available were included in the study. Asymptomatic patients (n=17) were identified on MRI scanning performed as part of a previously reported prospective study [6], and symptomatic patients (n=26) with MRI confirmation were enrolled in a natural history study of osteonecrosis. Fifty HIV-infected patients with no evidence of osteonecrosis on MRI

scanning, performed as part of the same study, and for whom serial plasma samples were available, served as the control group. The latter group had previously served as a control group in the analysis of coagulation abnormalities associated with osteonecrosis as noted above. Clinical and laboratory data have been previously published in reports on the prevalence, incidence, and natural history of osteonecrosis in HIV-infected patients [1, 6].

Concentrations of D-dimer in plasma samples frozen at -70°C were measured using the Sta Liatest D-Di immunoturbidimetric assay (Diagnostica Stago; detection limit of $0.22\ \mu\text{g/mL}$). Levels of CRP were determined by a highly sensitive immunonephelometry test (hsCRP, BN-II, Siemens Healthcare Diagnostics; detection limit of $0.17\ \text{mg/L}$). For the osteonecrosis cohort, samples were selected at the time of osteonecrosis diagnosis (± 2 months) and at time-points at least 6 months prior to and at least 6 months after the diagnosis time point. For control participants, samples were selected from the time of negative MRI imaging and 6 months later. Values below the detection limit of the assay were assigned a value of $0.21\ \mu\text{g/mL}$ for D-dimer and of $0.16\ \text{mg/L}$ for CRP.

Statistical Analysis

The primary objective was to compare D-dimer and CRP levels between patients with osteonecrosis and controls to look for an association between biomarker levels and osteonecrosis. D-dimer and CRP levels were compared at the time of MRI scan (diagnosis for osteonecrosis or documentation of normal femoral head for controls) between patients with osteonecrosis and controls using the Mann-Whitney U-statistic. To evaluate the relationship between biomarker levels over time and osteonecrosis, a line was fit to each subject's biomarker values over time. The slopes of each line, which describe the changes over time, were compared between the osteonecrotic patients and controls using a Mann Whitney U-statistic as well as a linear regression model that adjusted for viral load and total CD4 counts.

Results

Stored plasma samples were available for 43 osteonecrosis patients, 17 with asymptomatic and 26 with symptomatic disease. Samples were available from a time-point prior to the diagnosis of osteonecrosis for 26 patients (median - 6 months, range -29 to -6 months), at the time of diagnosis for 35 patients (median 0 months, time of diagnosis, range -2 to 2 months), and after diagnosis for 38 patients (median 7 months, range 6 to 35 months). All 50 control patients had samples available from the time of their negative MRI imaging and 5–7 months later (median 6 months).

The demographic characteristics of the patients included in the study are similar to those of the total cohort published previously [1, 6]. Selected demographic and clinic parameters of the participants at the time of MRI imaging are summarized in Table 1. The osteonecrosis cohort differed from the controls in the known duration of HIV infection (11.7 years vs. 8.8 years, $p=0.003$) and CD4 T-cell count ($465\ \text{cells/mm}^3$ vs. $686\ \text{cells/mm}^3$, $p=0.008$). No significant difference was seen in the percentage of patients receiving antiretroviral therapy (84% of the osteonecrosis cohort vs. 92% of the controls, $p=0.82$) or in the percentage of patients with suppressed viral loads (49% of the osteonecrosis cohort vs. 52% of the

controls, $p=0.83$). Antiretroviral regimens were similar between the two groups. Reflecting our clinic population at the time, 40% of the osteonecrosis patients and 60% of the controls had received prior interleukin-2 therapy ($p=0.05$). Of note, 19 (38%) of the control patients had received IL-2 in the year preceding their sample time-points, with interval from IL-2 ranging from 30 to 268 days. Six (14%) osteonecrosis patients had received IL-2 proximal to a sample time-point, ranging from 50 to 280 days before. As previously reported, osteonecrosis patients had a higher frequency of anti-cardiolipin IgG antibody levels greater than 23 units (32% vs. 10%, $p=0.01$).

Both D-dimer and CRP levels were significantly elevated at the time of diagnosis in the osteonecrosis cohort compared with controls (Table 1). For D-dimer, the median value was 0.32 $\mu\text{g/mL}$ in the osteonecrosis group compared to $<0.22 \mu\text{g/mL}$ in the control group ($p=0.016$), and for CRP the corresponding values were 2.52 mg/L and 1.23 mg/L ($p=0.003$). D-dimer and CRP levels were also significantly elevated in the osteonecrosis patients compared with the controls in the post-diagnosis period (Figure 1). These relationships remained significant when adjustments were made for HIV viral load and anti-cardiolipin antibody status. When examining changes in D-dimer and CRP over time by group, no significant differences were observed. Linear regression using data from the osteonecrosis patients with samples available for at least two time points ($n=36$) and all control patients (2 time points each), adjusted for HIV viral load at the first time-point, demonstrated that D-dimer levels rose from the pre-diagnosis to diagnosis for osteonecrosis patients, while remaining stable in the control cohort. CRP levels were stable over time (slopes were not different from zero) for both the osteonecrosis and control cohorts.

No difference was seen in D-dimer or CRP levels between the asymptomatic and symptomatic cohorts (data not shown).

Discussion

This study demonstrates that the occurrence of osteonecrosis in HIV-infected patients is associated with increased levels of two biomarkers that have been associated with poor clinical outcomes in HIV-infected patients. Compared to controls, patients who developed osteonecrosis had elevated levels of D-dimer and CRP at and after diagnosis. In osteonecrosis patients with samples available prior to diagnosis, D-dimer levels increased while CRP remained unchanged over time, whereas no significant change was noted in either biomarker in the control cohort. These data suggest that elevations in D-dimer are associated with the development of osteonecrosis. CRP elevations, however, predate the development of osteonecrosis, suggesting that at-risk patients have persistently higher levels of chronic inflammation, potentially as a result of ongoing immune activation, compared to the control cohort.

HIV-infected patients who develop osteonecrosis often have traditional risk factors associated with the development of osteonecrosis in other populations, but appear to be at increased risk in the context of these risk factors. Given the findings of this study, one potential explanation is that the development of osteonecrosis is multifactorial, and that chronic inflammation, as evidenced by persistently higher levels of CRP, creates an

environment in which, for example, use of corticosteroids results in a much greater risk of developing osteonecrosis. The direct role, if any, that chronic inflammation plays in the development of osteonecrosis cannot be determined from this study.

Non-traumatic osteonecrosis of the femoral head is pathologically characterized by venous hypertension and impaired venous circulation and is thought to be caused by intravascular coagulation and microcirculatory thrombosis, all of which result in compromised venous drainage. An association between thrombophilia and/or hypofibrinolysis with osteonecrosis in adults has been reported in numerous studies spanning more than 40 years. In 1961, Nilsson described a case of osteonecrosis of the hip associated with hypofibrinolysis [17]. Subsequently associations were described between PAI-1 levels and osteonecrosis [8, 18]. Depressed fibrinolysis was postulated to result in inadequate lysis of venous thrombi in bone, impaired bone venous circulation, and venous hypertension of bone.

Reports on D-dimer and CRP levels in patients with osteonecrosis are limited. In a recent report evaluating patients with idiopathic or corticosteroid-associated osteonecrosis, D-dimer levels were significantly higher in the latter, and tended to be higher in the former, compared to a similarly aged control group [19]. D-dimer levels were also significantly higher in Gaucher disease patients with osteonecrosis compared to those without osteonecrosis [20]. CRP levels were significantly elevated in a cohort of patients with non-traumatic osteonecrosis compared to age- and sex-matched controls [21]. However, a report of patients with severe acute respiratory syndrome (SARS) who subsequently developed osteonecrosis found that osteonecrosis patients differed from controls in levels of protein C, plasminogen activator inhibitor, plasminogen, and antithrombin III, but not in D-dimer levels [22]. In that study, samples were drawn after the diagnosis of osteonecrosis, and at least 6 months after treatment for SARS. [23]

Osteonecrosis thus appears to be another complication of HIV infection, like cardiovascular disease, that is a consequence of ongoing immune activation and inflammation. Whether immunosuppression contributes to the risk of osteonecrosis is uncertain; while the median CD4 count was lower in the osteonecrosis group, both groups had a relatively high median CD4 count, and the majority of patients in both groups had viral loads of <50 copies/ml. Biomarkers such as D-dimer and CRP can potentially identify HIV-infected patients at increased risk of osteonecrosis and other non-opportunistic complications, who may be good candidates for assessing therapeutic strategies that focus on reducing these non-opportunistic complications.

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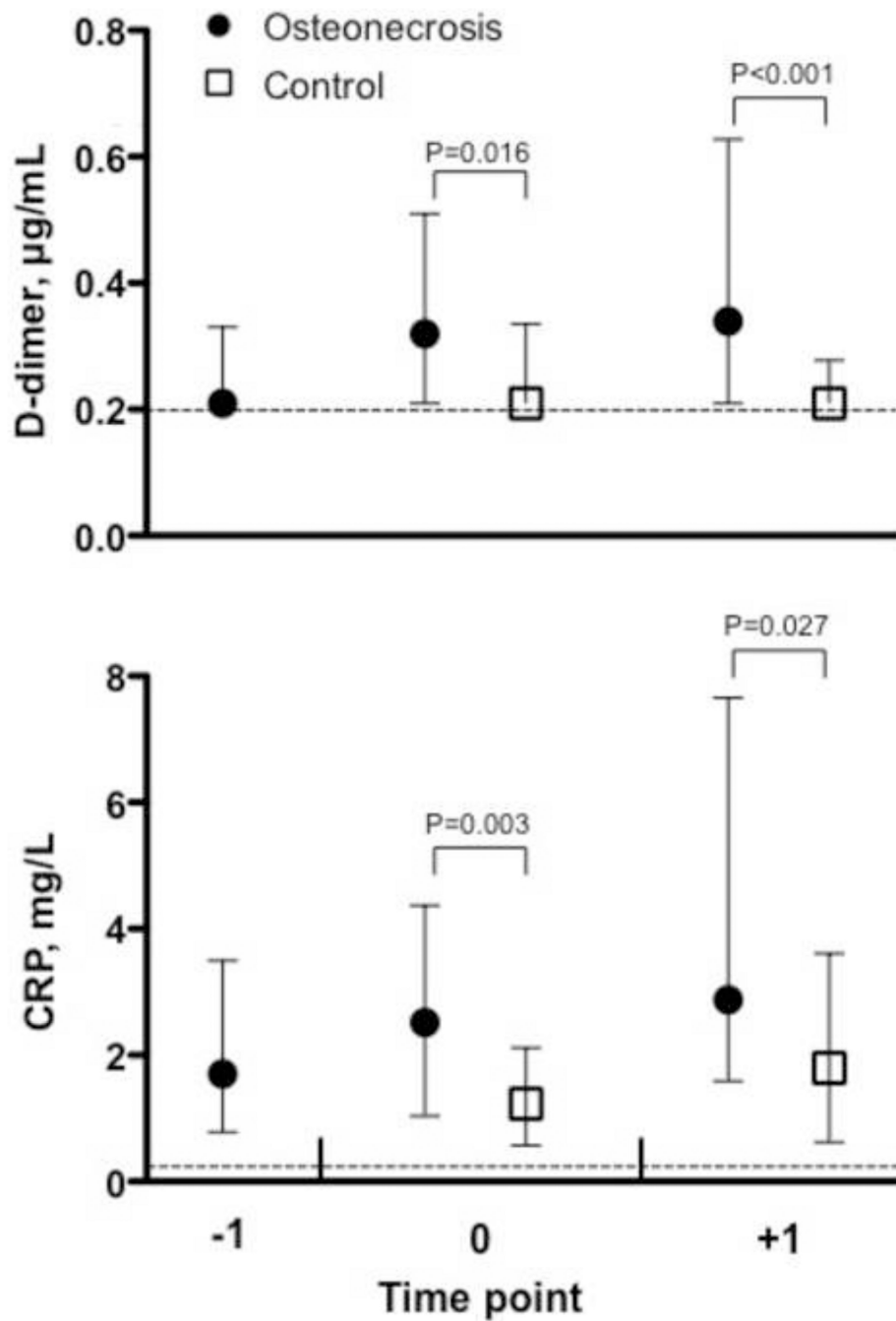


Figure 1.

D-dimer (top panel) and CRP (bottom panel) values for HIV-infected patients with osteonecrosis and controls. Medians with interquartile ranges are presented. For osteonecrosis patients, time point -1 represents data from before osteonecrosis diagnosis (6 to 29 months prior to diagnosis, n=26), time point 0 is at diagnosis (+/- 2 months, n=35) and time point +1 is after diagnosis (6 to 35 months after diagnosis, n=38). For control participants, time point 0 represents data from the time of negative MR imaging of the hips

and time point +1 is 6 months later (n=50). The dashed line indicates the lower limit of detection for the assays.

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Table 1

Demographic and clinical parameters for the osteonecrosis patients and controls.

	Osteonecrosis (n=43)	Control (n=50)	P-value
Age in years, median (range)	45.4 (20.8–61.8)	41.4 (28.3–70.0)	0.06
Male sex, n (%)	39 (91%)	43 (86%)	0.49
Duration of HIV at time of MRI in years, median (range)	11.7 (1.6–19.5)	8.8 (0.4–16.4)	0.003
CD4+ T-cell count nadir, cells/mm ³ , median (range)	143 (10–505)	213 (11–494)	0.42
CD4+ T-cell count, cells/mm ³ , median (range)	465 (12–1117)	686 (71–1705)	0.008
CD4+ T-cell percentage, median (range)	22 (1–51)	33 (10–57)	0.02
HIV viral load, copies/mL, median (range)	<50 (<50–242,844)	<50 (<50–306,953)	0.83
Number with HIV viral load < 50 copies/mL (%)	21 (49%)	26 (52%)	0.83
Receiving combination antiretroviral therapy, n (%)	36 (84%)	46 (92%)	0.82
Nucleoside Reverse Transcriptase Inhibitor, n (%)	36 (84%)	46 (92%)	
Non-nucleoside Reverse Transcriptase Inhibitor, n (%)	15 (35%)	19 (38%)	
Protease Inhibitors, n (%)	27 (63%)	35 (70%)	
Integrase inhibitor (raltegravir), n (%)	1 (2%)	0 (0%)	
History of Interleukin-2 Therapy, n (%)	17 (40%)	30 (60%)	0.05
Anticardiolipin antibody (ACA)			
Positive ACA IgM or IgG, n (%)	22/37 (60%)	21/49 (57%)	0.08
Positive ACA IgG, n (%)	20/37 (54%)	17/49 (34%)	0.04
ACA >23 Units, n (%)	12/37 (32%)	5/49 (10%)	0.01
Biomarker levels at time of diagnosis (osteonecrosis) or negative MRI (controls)			
D-dimer (µg/mL), median (range)	0.32 (<0.22–2.08)	<0.22 (<0.22–2.57)	0.016
CRP (mg/L), median (range)	2.52 (0.21–42.7)	1.23 (<0.17–24.4)	0.003
Biomarker levels at the time of diagnosis in persons with HIV viral load <50 copies/mL at the time of diagnosis (osteonecrosis, n=21) or negative MRI (controls, n=26)			
D-dimer (µg/mL), median (range)	0.32 (<0.22–1.97)	<0.22 (<0.22–2.57)	0.09
CRP (mg/L), median (range)	2.52 (0.43–42.7)	1.35 (<0.17–5.7)	0.01