Multifocal Osteonecrosis as a Severe Form of Glucocorticoid-Induced Osteonecrosis with Distinct Clinical and Proteomic Profiles

Kosuke Arita, Tomohiro Shimizu, Hotaka Ishizu, Yusuke Ohashi, Yutaro Sugawara, Daisuke Takahashi, Norimasa Iwasaki
Department of Orthopedic Surgery, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan
E-mail: kou.dr.2415124@gmail.com (K. Arita)

Disclosures: K. Arita (N), T. Shimizu (N), H. Ishizu (N), Y. Ohashi (N), Y. Sugawara (N), D. Takahashi (N), N. Iwasaki (N)

INTRODUCTION: Osteonecrosis (ON) is characterized by the death of osteocytes due to insufficient blood supply through various underlying mechanisms. While ON can affect multiple joints, including the shoulders, knees, and ankles, osteonecrosis of the femoral head (ONFH) is the most prevalent, with an estimated annual incidence of 10,000–20,000 new cases in the United States and 2,000–3,000 new cases in Japan. This number is expected to continue rising. Multifocal osteonecrosis, defined as ON occurring in three or more distinct anatomical sites, is a relatively rare condition, reported in approximately 3–11% of all ON cases. Recent studies utilizing whole-body MRI have revealed that asymptomatic osteonecrosis is more common than previously believed, with multiple ON lesions occurring at a much higher rate (20–86%) than earlier estimates suggested. The underlying conditions associated with multiple ON are diverse and include connective tissue diseases, hematological disorders, and respiratory infections. A common feature among these conditions is a history of glucocorticoid use; however, most existing studies and case reports have focused on single diseases, and there is no clear consensus on the role of glucocorticoid dosage or pulse therapy in ON development. In this study, we hypothesize that multifocal osteonecrosis involving three or more joints represents a more severe form of glucocorticoid-associated ON. To test this hypothesis, we aim to compare the clinical characteristics and comprehensive protein expression profiles, using proteome analysis, between patients with glucocorticoid-related ONFH and those with multifocal osteonecrosis.

METHODS: This study included 135 patients who underwent surgery for osteonecrosis of the femoral head (ONFH) at the Hokkaido University Hospital between April 2019 and December 2024. After excluding 28 patients with alcohol-induced ONFH who did not take glucocorticoids, 107 patients were included in the final analysis. The collected demographic and clinical data included sex; age at initial consultation; maximum daily glucocorticoid dose; history of glucocorticoid pulse therapy; alcohol consumption; smoking status; and comorbidities such as connective tissue disease, kidney disease, hematologic disease, and skin disorders. Patients with glucocorticoid-induced ONFH without multifocal osteonecrosis were classified into the GO group, whereas those with glucocorticoid-induced multifocal osteonecrosis were classified into the MGO group. Proteome analysis was conducted using three groups: GO, MGO, and osteoarthritis (OA) as controls. The OA group comprised patients with hip osteoarthritis who underwent surgery during the same period. Block randomization was performed based on sex and age (males in their 50s, females in their 50s, and males in their 60s) by selecting three patients from each group.

RESULTS: Multiple osteonecrosis occurred in 31 of 107 patients. The MGO group had a significantly younger mean age at the initial consultation than the GO group ($P = 0.021$). The MGO group also had a significantly higher mean maximum glucocorticoid dose than in the GO group ($P = 0.005$). A significantly higher proportion of patients in the MGO group had hematological diseases than those in the GO group ($P < 0.001$). Skin symptoms were significantly more prevalent in the MGO group ($P < 0.001$). Multivariate logistic regression analysis revealed that hematologic disease was the strongest predictor of multifocal osteonecrosis (odds ratio [OR], 14.51; 95% confidence interval [CI], 3.417–86.69; $P < 0.001$). Skin symptoms were also significantly associated with multifocal osteonecrosis (OR, 3.171; 95% CI, 1.046–10.44; $P = 0.046$). Proteome analysis revealed the diversity of the identified proteins was similar across the three groups; however, significant differences were observed in their protein expression profiles (Fig. 1). The fibrinolytic system factors PLG and SERPINC1, and the vascular protection factors SERPINA4 and ECM1 were significantly downregulated in the GO and MGO groups compared to the OA group ($P<0.05$), especially SERPINA4 and ECM1 were significantly downregulated in the MGO group compared to the GO group ($P<0.05$). The blood coagulation factors FGB, FGG, and VWF were significantly upregulated in the MGO group compared to the GO and the OA group ($P<0.05$, Fig. 2).

DISCUSSION: This study demonstrates that multifocal osteonecrosis, involving three or more joints, represents a more severe form of glucocorticoid-associated osteonecrosis with distinct clinical and proteomic characteristics. Compared with patients with glucocorticoid-induced ONFH without multifocal involvement, those with multifocal disease were significantly younger at diagnosis and had received higher maximum glucocorticoid doses. Hematologic diseases and skin symptoms were identified as independent risk factors, suggesting distinct clinical backgrounds. Proteomic analysis revealed significantly reduced levels of key fibrinolytic proteins, including PLG and SERPINC1, which are essential for preventing excessive clot formation and maintaining blood flow [1,2]. Their reduction suggests impaired thrombolytic activity, which may contribute to osteonecrosis. Conversely, fibrinogen components (FGB, FGG) and VWF were significantly elevated in the MGO group compared with both GO and OA groups, supporting enhanced coagulation that may exacerbate bone ischemia and osteonecrosis, consistent with previous reports implicating enhanced coagulation in ONFH pathogenesis [3–5]. Additionally, vascular protective factors ECM1 and SERPINA4 were significantly lower, while inflammatory marker SERPINA3 was higher in the MGO group compared with both GO and OA groups. ECM1 contributes to extracellular matrix stability and vascular integrity [6], SERPINA4 regulates vascular function [7], and SERPINA3 is linked to inflammation and tissue remodeling [8]. These findings suggest that enhanced coagulation, reduced fibrinolysis, and impaired vascular protection contribute to the pathogenesis of glucocorticoid-induced multifocal osteonecrosis.

CLINICAL RELEVANCE: This study adds important new insights by identifying hematologic disease and skin manifestations as independent predictors of glucocorticoid-induced multifocal osteonecrosis. Proteomic analysis further implicates enhanced coagulation, reduced fibrinolysis, and impaired vascular protection in its pathogenesis, suggesting potential targets for early diagnosis and prevention.

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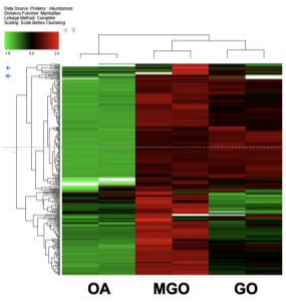

Fig 1. To identify the factors associated with the development of multifocal osteonecrosis, preoperative serum samples pooled per group were subjected to shotgun proteomic analyses. Protein expression profiles across groups.

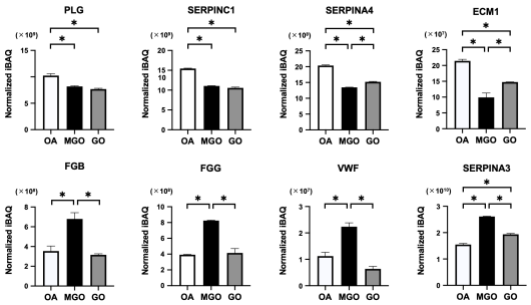

Fig 2. Comparison of protein expression levels among OA (osteoarthritis patients), MGO (glucocorticoid-induced multifocal osteonecrosis (MGO), and GO (glucocorticoid-induced osteonecrosis of the femoral head (ONFH) without multifocal osteonecrosis). * $P < 0.05$ indicates a significant difference between the two groups. PLG, plasminogen; SERPINC1, antithrombin-III; SERPINA4, kallistatin; ECM1, extracellular matrix protein 1; FGB, fibrinogen beta chain; FGG, fibrinogen gamma chain; VWF, von Willebrand Factor, SERPINA3, alpha-1-antichymotrypsin.