Postoperative Changes in the Systemic Inflammatory Milieu in Older Surgical Patients

Lauren Shih, MD[1,2], Nil Guler, MD[3], Daneyal Syed, MD[3],
William Hopkinson, MD[3], Kyra N. McComas, BS[1],
Amanda Walborn, MS[3], Debra Hoppensteadt, PhD[3],
Jawed Fareed, PhD[3], and Matthew T. Rondina, MD, MS, FAHA[1,4,5]

Abstract

Dysregulated inflammation is a central component of wound healing following surgery. We prospectively enrolled older patients (n = 25, age 65 $\pm$ 7 years) undergoing elective total knee arthroplasty or total hip arthroplasty secondary to advanced osteoarthritis (OA) and healthy controls (n = 48). Inflammatory, proangiogenic (vascular endothelial growth factor [VEGF], monocyte chemoattractant protein-1 [MCP-1], and interleukin-8 [IL-8]), and antiangiogenic (interferon γ [IFN-γ] and IL-4) factors were measured using a high-sensitivity biochip. Patients with OA had significantly higher baseline VEGF (10.5 $\pm$ 1.2 pg/mL vs 4.8 $\pm$ 0.2 pg/mL, $P < .001$), MCP-1 (130.6 $\pm$ 7.7 pg/mL vs 88.6 $\pm$ 3.9 pg/mL, $P < .0001$), and IL-8 (4.0 $\pm$ 0.5 pg/mL vs 2.6 $\pm$ 0.1 pg/mL, $P < .05$). Postoperatively, the levels of VEGF (10.5 $\pm$ 1.2 pg/mL vs 18.8 $\pm$ 1.5 pg/mL, $P < .001$) and MCP-1 (130.6 $\pm$ 7.7 pg/mL vs 153.1 $\pm$ 11.5 pg/mL, $P < .05$) increased significantly. Baseline and postoperative MCP-1 levels correlated positively and significantly with age. The levels of IFN-γ and IL-4 (which has anti-inflammatory properties) did not significantly differ at baseline in patients with OA compared to controls and did not significantly rise postoperatively. We conclude that systemic levels of pro-inflammatory and angiogenic proteins are increased in patients with OA and rise further postoperatively, while proteins that restrain inflammation and angiogenesis do not coordinately rise. These findings implicate imbalance in inflammatory pathways in OA that may contribute to its pathobiology.

Keywords

surgery, aging, inflammation, angiogenesis, obesity

Introduction

Osteoarthritis (OA) is a disease of major public health impact worldwide and the most common chronic joint disease in senior adults.[1] The mechanisms underpinning OA are incompletely understood. However, an accumulating body of knowledge demonstrates that OA is associated with an injurious pro-inflammatory milieu within the joint structure contributing to cartilage destruction.[2] Cartilage destruction has been proposed as a result of chondrocyte apoptosis. As a highly regulated and coordinated process of cell death, apoptosis has been linked to development, homeostasis, and aging[1] and may be implicated in the production of inflammatory signals. Monocyte chemo-attractant protein-1 (MCP-1) is one such signal of inflammation that has been shown to be expressed after injury. In knee OA, MCP-1 functions to recruit and activate synovial mesenchymal stem cells (sMPCs) while also inhibit their differentiation for chondrogenesis. Simultaneously, MCP-1 triggers transcriptional upregulation in sMPCs for increased

MCP-1 expression, which could contribute to the lack of internal self-repair in OA.[3]

In advanced cases of OA involving the knee and hip joints, orthopedic surgery is commonly required. Regulated vascular responses and wound healing are critical for patients recovering from these surgical procedures. Disruption of these host

[1] Molecular Medicine Program, University of Utah, Salt Lake City, UT, USA
[2] Department of Internal Medicine, University of Washington, Seattle, WA, USA
[3] Department of Pathology, Loyola University Medical Center, Maywood, IL, USA
[4] Department of Pathology, George E. Wahlen VAMC Geriatric Research, Education and Clinical Center, Salt Lake City, UT, USA
[5] Department of Internal Medicine, University of Utah, Salt Lake City, UT, USA

Corresponding Author:
Matthew T. Rondina, Eccles Institute of Human Genetics, University of Utah Health Sciences Center, Bldg. 533, Rm 4220A, Salt Lake City, UT 84112, USA.
Email: matthew.rondina@hsc.utah.edu

responses (or impairments and dysregulation at the tissue, cellular, or systemic level) may result in poor healing, the need for repeat surgery, and complications such as infection, chronic wounds, and disability. Past studies suggest that MCP-1 stimulates angiogenesis, as well as tumor necrosis factor α (TNF-α), interleukin-1β (IL-1β), and IL-8, primarily via oxidative stress, endoplasmic reticulum stress, and autophagy.[4]

Current treatments for OA tend to focus on symptomology, but there is little knowledge about therapeutic targets that contribute to cartilage destruction, including inflammatory and angiogenic factors. Thus, new knowledge underlying the pathobiology of OA is necessary to identify or improve therapeutic strategies. Published studies have found that systemic levels of IL-8 and vascular endothelial growth factor (VEGF) are increased in patients with knee OA[5,6] and VEGF levels correlate with severity of disease in the joint.[7] Nevertheless, whether systemic upregulation of vascular and inflammatory proteins is present in patients with OA at baseline and immediately following surgery remains unexamined. Here, we sought to explore this potential upregulation using a highly sensitive protein bioarray in a prospective study of patients with advanced OA undergoing joint replacement surgery.

Methods

Human Participants

Patients with OA (n = 25) were recruited from a single, tertiary, academic medical center using an institutional review board–approved protocol. Osteoarthritis was confirmed radiologically in all patients. All patients provided informed consent before any study procedures were initiated. Inclusion criteria were consenting adults (aged 21 years and older) undergoing elective total knee arthroplasty (TKA) or total hip arthroplasty (THA). Patients with a history of thrombosis, renal or hepatic dysfunction, surgery within 30 days, pregnancy, acute coronary syndrome, infection, or severe hypertension (≥160/95 mm Hg) were excluded. All patients were American Society of Anesthesiologists Physical Class I or II. The same 2 surgical teams from an academic medical center performed all surgeries. An independent study examining platelet activation patterns and associations with venous thromboembolism (VTE) in patients from this study has previously been published.[8]

Intermittent compression devices and Thrombo-Embolic Deterrent hose were applied to all patients postoperatively. No patients received preoperative anticoagulation. Postoperatively, all patients received warfarin for VTE prophylaxis. Warfarin was initiated the evening of surgery and adjusted to achieve an International Normalized Ratio goal of 2 to 3.[9] Preoperative aspirin use (81 to 162 mg daily) was continued postoperatively if recommended by the patient's physician and Non-steroidal antiinflammatories were discontinued preoperatively. Patients were followed prospectively for 35 days postoperatively for the development of any symptomatic, clinically evident VTE. For comparison, plasma from apparently healthy volunteers free from OA (n = 48) were included.

Compression Ultrasonography

As part of the primary study protocol,[8] comprehensive bilateral, lower extremity duplex compression ultrasonography (CUS) was performed by registered vascular technologists for all patients approximately 2 to 3 days after surgery. A second, follow-up CUS was also performed about 14 days postoperatively. Details on this protocol have been published previously.[8] Briefly, the deep veins of the thigh and calf were examined in a sequential manner in 1- to 2-cm increments. The following deep venous segments were investigated: the common femoral vein, the mid and distal femoral vein, the popliteal vein, the trifurcation of the deep calf veins, the posterior tibial veins, the gastrocnemius veins, the soleal sinus vein, and the peroneal veins. Lack of venous compressibility with the ultrasound transducer held in a transverse position to the vein was interpreted as a positive study. Color flow and pulsed wave Doppler analyses was used for confirmation. A board-certified vascular surgeon interpreted all ultrasounds. Proximal deep vein thrombosis (DVT) was defined as an acute appearing thrombosis involving the popliteal and/or more proximal lower extremity deep vein segments. Distal DVT was defined as an acute appearing thrombosis in any deep vein segment distal to the popliteal vein. For the end point of DVT, events were captured by CUS up until postoperative day (POD) 35.

Protein Measurements and Biochip Assay

Platelet-poor plasma was harvested from patients with OA by centrifugation (150*g* for 20 minutes at 20°C) immediately preoperatively (baseline, BL) and then again on the morning of POD 1. Plasma was immediately frozen at −80°C until used for protein assays. A Randox Cytokine and Growth Factors High-Sensitivity Array assay kit was used to profile IL-2, IL-4, IL-6, Il-8, IL-10, VEGF, interferon γ (IFN-γ), TNF-α, IL-1α, IL-1β, MCP-1, and epidermal growth factor (EGF; Randox, London, United Kingdom). This allowed quantification of all factors in a single patient sample simultaneously using a sandwich chemiluminescent immunoassay. Briefly, each biochip provided in the kit contained 12 testing regions, each with a different immobilized antibody specific to a different cytokine. The chip was incubated with the patient sample. After washing, conjugate, consisting of horseradish peroxidase-labeled, analyte-specific antibodies, was incubated with the chip. Increased level of a bound cytokine increased binding of conjugate and thus the chemiluminescent signal emitted upon addition of the signal reagent. The luminescent signal generated from each region of the biochip was translated into analyte concentration by the Randox evidence investigator using a calibration curve generated based on controls of known concentration.

Statistical Analyses

Data were examined for normality using skewness and kurtosis tests. Comparisons were made using the Student *t* test or Wilcoxon rank sum test (for continuous variables) and the

Table 1. Characteristics of the Patients With OA Undergoing Surgery.[a]

Characteristic	
Age, years	65 (7)
Male (gender), n (%)	11 (44%)
BMI, kg/m^{2}	31 (7)
Obesity, n (%)	12 (48%)
Medical history	
Diabetes, n (%)	6 (24%)
Coronary artery disease, n (%)	1 (4%)
Preoperative medications	
Anti-inflammatory, n (%)	14 (56%)
Aspirin, n (%)	10 (40%)
Plavix, n (%)	0 (0%)
Statin, n (%)	9 (36%)
Surgical procedure and postoperative outcomes	
Total knee arthroplasty, n (%)	17 (68%)
Total hip arthroplasty, n (%)	8 (32%)
Any deep vein thrombosis, n (%)	9 (36%)
Proximal DVT, n (%)	3 (12%)
Distal DVT, n (%)	6 (24%)

Abbreviations: BMI, body mass index; DVT, deep vein thrombosis; OA, osteoarthritis.
[a]Data represent the mean (standard deviation, SD), unless otherwise indicated.

χ^2 or Fisher exact test (for categorical variables), as appropriate (STATA v11.0). Central tendency data are reported as the mean (SD) or median (interquartile range) if the distribution was skewed. Correlation analyses between continuous variables were presented as the Pearson correlation coefficient. A 2-sided *P* value <.05 was considered statistically significant.

Results

Demographics, medications, and thrombotic outcomes are shown in Table 1. The average age was 65 years, consistent with OA being more prevalent in aging.[1] Nearly half of our patients (48%) were obese. As is common in patients with severe OA and degenerative joint disease, anti-inflammatory agents were used in more than 50% of patients. Approximately two-thirds of patients with OA underwent TKA, with the remainder undergoing THA. Distal DVT was not uncommon as we have previously reported and most (7/9 or 77.7%) of the thrombotic events were identified on the first CUS performed in patients 2 to 3 days after surgery.[8] Two patients had DVT identified on PODs 8 and 31. Baseline and postoperative laboratory values are shown in Table 2. As expected, the hemoglobin and platelet count decreased significantly postoperatively, while the white blood cell count and C-reactive protein levels rose significantly, consistent with a systemic inflammatory insult.

We initially examined baseline (eg, preoperative) inflammatory proteins in patients with OA compared to healthy control participants. We identified that at baseline, patients with OA had significantly higher levels of VEGF (10.5 ± 1.2 vs 4.8 ± 0.2 pg/mL, *P* < .001; Figure 1A). Consistent with upregulation of inflammation and angiogenesis,[10] we noted

Table 2. Preoperative and Postoperative Laboratory Values in Surgery Patients.[a]

Labs	Preoperative	Postoperative Day 1 (POD1)	*P* Value
Hemoglobin, g/dL	13.7 (1.1)	11.4 (1.4)	<.0001
White blood cells, K/μL	6.9 (2.5)	9.8 (3.8)	<.0001
Hematocrit, %	39.7 (3.8)	33.0 (4.2)	<.0001
Platelets, K/μL	272 (59)	228 (52)	<.0001
C-reactive protein, mg/L	6.51 (14.86)	18.83 (17.90)	<.0001

[a]Data represent the mean (standard deviation, SD), unless otherwise indicated.

significantly higher levels of MCP-1 (130.6 ± 7.7 pg/mL vs 88.6 ± 3.9 pg/mL, *P* < .0001; Figure 1B) and IL-8 (4.0 ± 0.5 pg/mL vs 2.6 ± 0.1 pg/mL, *P* < .05; Figure 1C) compared to healthy control participants.

Postoperatively, the levels of VEGF (10.5 ± 1.2 vs 18.8 ± 1.5 pg/mL, *P* < .001) and MCP-1 (130.6 ± 7.7 pg/mL vs 153.1 ± 11.5 pg/mL, *P* < .05) significantly increased in patients with OA (Figure 2A and B). IL-8 also increased postoperatively, although statistical significance was not achieved (4.0 ± 0.5 pg/mL vs 4.7 ± 0.6 pg/mL, *P* = .051; Figure 2C). The EGF, which stimulates cell growth, proliferation, and differentiation, was about 4-fold higher at baseline in patients with OA compared to healthy controls (5.6 ± 1.7 pg/mL vs 1.4 ± 0.2 pg/mL, *P* = .0046) but did not change significantly postoperatively (5.6 ± 1.7 pg/mL vs 2.9 ± 0.6 pg/mL, *P* = .33).

As MCP-1 has been implicated in aging,[11] where angiogenesis is dysregulated,[9] we next explored whether plasma MCP-1 levels correlated with age in patients with OA. As shown in Figure 3A and B, both baseline and postoperative levels of MCP-1 significantly and positively correlated with age. Obesity with inflammation has also been shown to correlate with OA and is a substantial risk factor,[12] so we further explored whether plasma MCP-1 levels correlate concordantly. We examined our surgical cohort but did not find any significant association between MCP-1 levels and obesity (data not shown). Anti-inflammatories were used in 57% (14/25) of our patients (Table 1). However, neither baseline nor postoperative levels of MCP-1, IL-6, IL-8, IL-10, or VEGF were significantly affected by the use of anti-inflammatories.

As we identified upregulation of circulating pro-angiogenic and pro-inflammatory proteins in patients with OA, we also sought to determine whether antiangiogenic and anti-inflammatory molecules were concordantly increased. Interestingly, the levels of the anti-angiogenic factors IFN-γ and IL-4 were generally low, did not differ between patients with OA and controls, and did not change significantly on POD 1 (Figure 4A and B). As part of the bioarray, we also measured IL-1, IL-2, IL-10, IL-6, and TNF-α. Baseline levels of these proteins did not differ between patients with OA and healthy controls (not shown). Of these 5 proteins, only IL-6 and IL-10 significantly rose postoperatively, with IL-6 levels rising 45-fold (2.3 ± 0.4 pg/mL vs 105.0 ± 12.2 pg/mL, *P* < .0001) and IL-10 levels rising more than 2-fold (0.64 ± 0.1 pg/mL vs 1.5 ± 0.2 pg/mL, *P* = .0003).
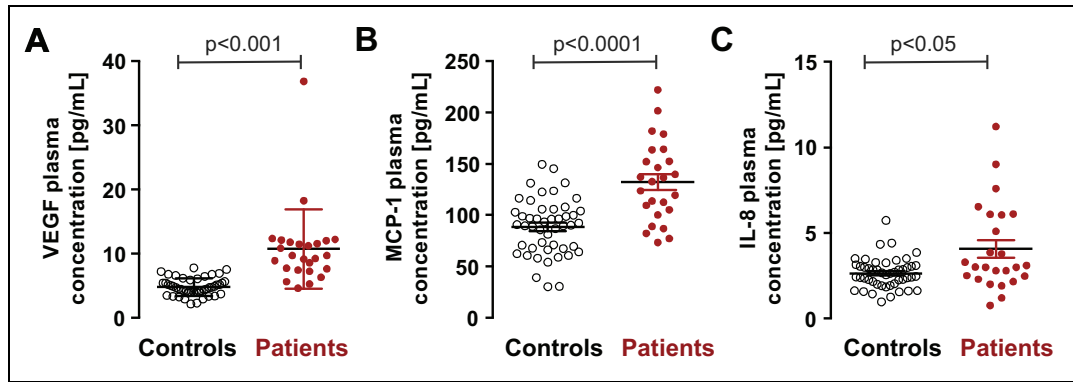
Figure 1. Plasma inflammatory and angiogenesis proteins are increased at baseline in patients with OA. Plasma concentrations of (panel A) VEGF, (panel B) MCP-1, and (panel C) IL-8 were measured in healthy control participants (n = 48) or at baseline, preoperatively, in patients with OA (n = 25). IL-8 indicates interleukin 8; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis; VEGF, vascular endothelial growth factor.
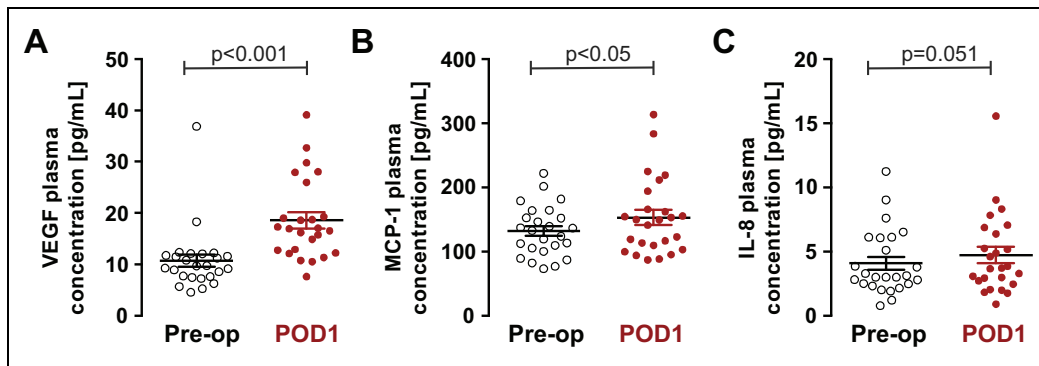
Figure 2. Plasma inflammatory and angiogenesis proteins increase postoperatively in patients with OA. Plasma concentrations of (panel A) VEGF, (panel B) MCP-1, and (panel C) IL-8 in patients with OA preoperatively (preop) or on postoperative day 1 (POD 1; n = 25). IL-8 indicates interleukin 8; MCP-1, monocyte chemoattractant protein-1; OA, osteoarthritis; VEGF, vascular endothelial growth factor.
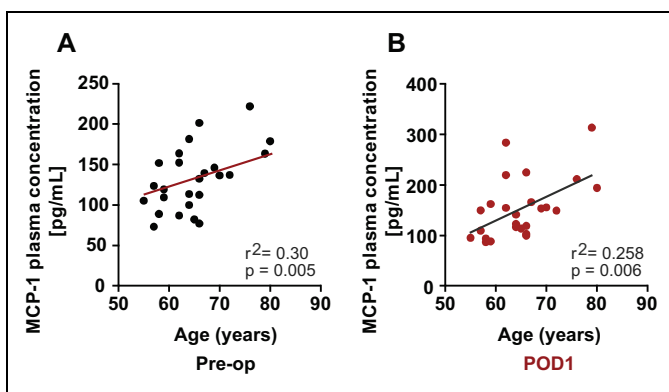
Figure 3. Plasma MCP-1 levels correlate with age in patients with OA. Correlation between MCP-1 plasma levels and age in OA patients preoperatively (preop, panel A) and on postoperative day 1 (POD 1, panel B; n = 25). MCP-1 indicates monocyte chemoattractant protein-1; OA, osteoarthritis.
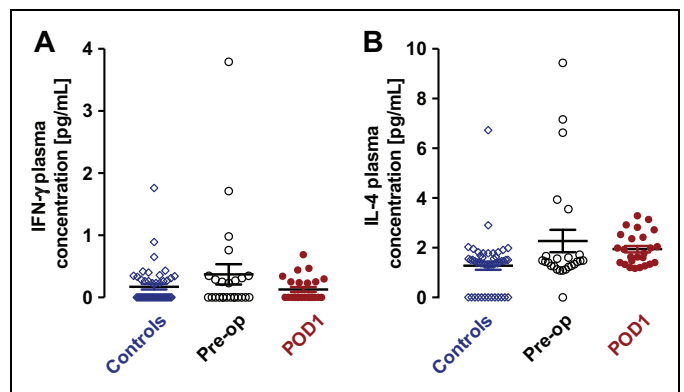
Figure 4. Plasma IFN-γ and IL-4 levels do not rise postoperatively in patients with OA. Plasma concentrations of (panel A) IFN-γ and (panel B) IL-4 in patients with OA preoperatively (preop) or on postoperative day 1 (POD 1; n = 25). IFN-γ indicates interferon-gamma; IL-4, interleukin-4; OA osteoarthritis.

Deep vein thrombosis occurred in 36% of patients in our cohort (Table 1). We examined whether postoperative changes in MCP-1, IL-6, IL-8, IL-10, or VEGF correlated with thrombotic outcomes. Compared to preoperative levels, changes in IL-6 and VEGF on POD 1 trended higher in patients who developed DVT compared to patients who did not develop

thrombosis but did not reach statistical significance (IL-6 fold-change: 89.2 ± 25.6 vs 49.8 ± 9.9, $P = .10$ and VEGF fold-change: 2.36 ± 0.3 vs 1.77 ± 0.2, $P = .11$). There were no differences in MCP-1, IL-8, or IL-10 between patients with or without DVT.

Discussion

Our findings build upon and extend prior studies in this area. Epidemiological studies have identified increased circulating levels of IL-8 and VEGF in patients with knee OA[5,6] and VEGF levels correlate with severity of disease in the joint.[7] Here, we show that in addition to VEGF, plasma levels of MCP-1 are increased significantly at baseline in patients with OA compared to healthy controls. We further demonstrate that VEGF and MCP-1 levels systemically increased in patients with OA following knee and hip surgery, with a concordant trend in IL-8. Furthermore, we have demonstrated a correlation between MCP-1 and age, which is a significant factor in the pathogenesis of OA.

Postoperative increases in IL-6 and IL-10 in patients with OA suggest a dysregulated homeostatic response, since IL-6 is primarily pro-inflammatory and IL-10 is anti-inflammatory. This could be a result of anti-inflammatory medication effects before, during, and after surgery. However, it is interesting that TNF-α failed to at least be elevated at baseline, since it has been linked to increased hypoxia-inducible factor-2α binding to FasL, which is a member of the TNF receptor family, and has been shown to contribute to chondrocyte apoptosis,[13] in parallel with MCP-1 inhibition of chondrogenesis.[3] This finding contributes to the complexity of the immunomodulatory functions of the human body but may provide clues into how the dysregulated immune response of OA may be managed.

With the increase in circulating proangiogenic and pro-inflammatory proteins in postsurgical patients with OA, the lack of increase in antiangiogenic factors IFN-γ and IL-4 suggests loss of homeostasis control and shift toward a pro-inflammatory milieu. This expands other studies that have found a pro-inflammatory phenotype in patients with OA[12] by adding a component of dysregulated homeostasis and angiogenesis. Moreover, our correlations with age are supported by the current literature.[1] However, our surgical cohort did not have a significant correlation between pre- and postoperative MCP-1 levels and obesity or body mass index, despite past research suggesting a correlation between OA and obesity.[12] As such, while MCP-1 may not be a target for obesity, it could be implicated in targeted therapy for aging and OA. Yet systemic IL-6, leptin, resistin, CCL7 (previously MCP-3), and nerve growth factor have been found to be elevated in patients with OA, as well as a possible phenotype of increased synovial IL-17 that could be used for therapy targeting for this subset of patients.[12] Thus, the identification of the pro-inflammatory (MCP-1 and IL-8) and proangiogenic (VEGF) factors in our study could provide potential therapeutic targets for patients with OA that go beyond symptom management.

Strengths of our study include the prospective design, serial and comprehensive assessment of circulating proteins, inclusion of a healthy cohort for comparison, implications for OA therapy, and a deeper understanding of the complex inflammatory and angiogenic context of OA. However, our study has a small sample size and cannot draw firm conclusions regarding how dysregulated inflammatory pathways contribute to the pathobiology of OA. We also did not examine levels of inflammatory proteins beyond POD 1 and thus changes in plasma protein levels postoperatively may reflect changes due to surgical intervention as well as inflammation associated with OA. While not the primary focus of our study, we also captured clinical thrombotic outcomes prospectively. The MCP-1 and VEGF increases postoperatively were higher in patients who subsequently developed DVT but did not reach statistical significance. This may be due to the small sample size and insufficient power to correlate plasma proteins with these events.

Conclusion

In conclusion, circulating levels of the proangiogenic proteins VEGF, MCP-1, and IL-8 were increased in patients with OA at baseline compared to healthy controls and VEGF and MCP-1 rose significantly postoperatively, with a similar trend in IL-8, while the antiangiogenic factors IFN-γ and IL-4 remained unchanged. Baseline MCP-1 levels correlated positively and significantly with age, suggesting an interaction between aging and the pathophysiology of OA. These studies also illustrate the power of a sensitive and high-throughput profiling assay for clinical studies.

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