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Men and Women Differ in the Biochemical Composition of Platelet-Rich Plasma

Grace Xiong,*† BA, Nithya Lingampalli,†‡ BS, Jayme C.B. Koltsov,* PhD, Lawrence L. Leung,†§ MD, Nidhi Bhutani,* PhD, William H. Robinson,†‡ MD, PhD, and Constance R. Chu,*†‖ MD
Investigation performed at Stanford University, Stanford, California, USA, and the Veterans Affairs Palo Alto Health Care System, Palo Alto, California, USA

Background: Autologous platelet-rich plasma (PRP) is widely used for a variety of clinical applications. However, clinical outcome studies have not consistently shown positive effects. The composition of PRP differs based on many factors. An improved understanding of factors influencing the composition of PRP is important for the optimization of PRP use.

Hypothesis: Age and sex influence the PRP composition in healthy patients.

Study Design: Controlled laboratory study.

Methods: Blood from 39 healthy patients was collected at a standardized time and processed into leukocyte-poor PRP within 1 hour of collection using the same laboratory centrifuge protocol and frozen for later analysis. Eleven female and 10 male patients were ''young'' (aged 18-30 years), while 8 male and 10 female patients were ''older'' (aged 45-60 years). Thawed PRP samples were assessed for cytokine and growth factor levels using a multiplex assay and enzyme-linked immunosorbent assay. The platelet count and high-sensitivity C-reactive protein levels were measured. Two-way analysis of variance determined age- and sex-based differences.

Results: Platelet and high-sensitivity C-reactive protein concentrations were similar in PRP between the groups (P = .234). Male patients had higher cytokine and growth factor levels in PRP compared with female patients for inflammatory cytokines such as interleukin–1 beta (IL-1β) (9.83 vs 7.71 pg/mL, respectively; P = .008) and tumor necrosis factor–alpha (TNF-α) (131.6 vs 110.5 pg/mL, respectively; P = .048); the anti-inflammatory IL-1 receptor antagonist protein (IRAP) (298.0 vs 218.0 pg/mL, respectively; P < .001); and growth factors such as fibroblast growth factor–basic (FGF-basic) (237.9 vs 194.0 pg/mL, respectively; P = .01), platelet-derived growth factor (PDGF-BB) (3296.2 vs 2579.3 pg/mL, respectively; P = .087), and transforming growth factor–beta 1 (TGF-β1) (118.8 vs 92.8 ng/mL, respectively; P = .002). Age- but not sex-related differences were observed for insulin-like growth factor–1 (IGF-1) (P < .001). Age and sex interaction terms were not significant. While mean differences were significant, there was also substantial intragroup variability.

Conclusion: This study in healthy patients shows differences in the composition of PRP between men and women, with sex being a greater factor than age. There was also proteomic variability within the groups. These data support a personalized approach to PRP treatment and highlight the need for a greater understanding of the relationships between proteomic factors in PRP and clinical outcomes.

Clinical Relevance: Variability in the proteomic profile of PRP may affect tissue and clinical responses to treatment. These data suggest that clinical studies should account for the composition of PRP used.

Keywords: platelet-rich plasma; cytokines; growth factors; sex differences

The clinical use of autologous platelet-rich plasma (PRP) in orthopaedics has exponentially increased in the past decade. First used in orthopaedic sports medicine to treat tendinopathy,[39] PRP use has expanded to include a wide variety of pathological conditions such as muscle strain, augmentation of surgical repair, knee osteoarthritis,[20,21,30,56] and osteomyelitis.[46,72] The benefits of PRP are hypothesized to derive from the regenerative effects of growth factors released by platelets as well as anti-inflammatory effects that help tissue healing and relieve pain.[23,37,51] Early in vitro and animal studies have reported improved effects on bone regeneration,[29,44] chondrocyte proliferation,[1] mesenchymal differentiation,[70] extracellular matrix metabolism,[2] and anabolic gene expression.[61] Clinical outcome studies, however, have not consistently shown positive effects.[22,41]

Several theories have been proposed to explain the wide variation in PRP outcomes. One area of focus has been variability in PRP produced using devices from different manufacturers in which wide disparities in the platelet count, white blood cell count, and some growth factors have

been reported.[7,11,17,49,66] However, equipment and protocols are not the only source of variation, and it has been suggested that even samples from the same patient prepared using the same system or at different times may yield different results.[7,25] Given the autologous source of PRP, differences between patients are also likely to be a significant source of proteomic variation that may affect tissue responses and treatment efficacy.

Age and sex affect biology and may also be important variables that influence the composition of PRP. In a study evaluating the chondrogenic potential of human bone marrow–derived mesenchymal stem cells, age influenced chondroid differentiation in response to transforming growth factor–beta 1 (TGF-β1) in male participants but did not show a similar effect in female participants.[53] Biological differences in healing potential and inflammation related to age and sex may be reflected in the biochemical composition of PRP. This study was therefore performed to test the hypothesis that the composition of PRP prepared from healthy patients differs by age and sex.

METHODS

Thirty-nine healthy patients (body mass index [BMI] <25 kg/m^2; with no history of orthopaedic problems or surgery; and who were not taking medications in the nonsteroidal anti-inflammatory class or anti-platelet class, aspirins, or any supplements for musculoskeletal health such as chondroitin sulfate) were recruited according to institutional review board–approved protocols and provided informed consent. Volunteers in a "young" group aged 18 to 30 years (10 male, 11 female) and an "older" group aged 45 to 60 years (8 male, 10 female) were recruited. The 15-year age gap between the groups was instituted in the study design to facilitate the observation of age-related differences. A 25 mL sample of whole blood was obtained by a licensed clinical phlebotomist using a standard venipuncture technique between 7 AM and 9 AM.

Preparation of PRP

Blood drawn into a vacutainer containing anticoagulant citrate dextrose solution (Becton Dickinson) was processed within 1 hour of collection at room temperature in a standard laboratory centrifuge (Beckman Coulter) according to a standard PRP preparation protocol established by the study hematologist to generate leukocyte-poor PRP.[45] Briefly, the fresh whole blood was centrifuged at 180g for 10 minutes, after which the plasma fraction containing the platelets was carefully aspirated, avoiding the region immediately above the buffy coat. The resulting PRP was then subaliquoted into cryovials, taking care to gently invert the plasma tube periodically to allow the resuspension of platelets. The cryovials were stored at –80°C until analysis.

Hematological Analysis

Aliquots of whole blood were assessed for complete blood counts with differentials, high-sensitivity C-reactive protein levels, and hemoglobin A1c levels by the hospital clinical laboratory according to standard protocols. Platelet counts for the PRP samples were determined using a hemocytometer at 20× magnification using previously validated methods for counting platelets and white blood cells.[34,35]

Cytokine and Growth Factor Analysis

To assess the cytokine and growth factor composition of the PRP samples, frozen samples of PRP were thawed at room temperature for 30 minutes; thus, one freeze-thaw cycle was used to activate platelets.[9] Thawed samples were used immediately and samples were briefly vortexed to ensure sample homogeneity before analysis. All samples were run on plates of the same lot number processed at the same time. Multiplex analysis of 27 cytokines was performed using the Bio-Plex Pro Human Cytokine 27-plex Assay (Bio-Rad Laboratories) run on a Luminex 200 System (Luminex Corp) according to the manufacturer's instructions, with the exception that the proprietary Bio-Rad assay dilution buffer was modified to contain reagents that reduce the effect of heterophilic antibodies in multiplex immunoassays, as previously described.[38,63] Data processing was performed with Bio-Plex Manager 5.0 Software (Bio-Rad Laboratories), and plasma concentrations (pg/mL) were interpolated from standard curves for each respective cytokine or chemokine. Blank wells were run concurrently and the resulting median fluorescence intensity subtracted from sample median fluorescence intensity values to ensure no background reactivity. Insulin-like growth factor–1 (IGF-1) and TGF-β1 levels were assessed by an enzyme-linked immunosorbent assay using the manufacturer's protocols (R&D Systems). For all assays, samples were run in duplicate.

Statistical Analysis

Statistical analyses were performed using Stata SE version 13.0 for Mac (StataCorp). Demographic differences in age or BMI and differences in laboratory values (whole blood platelet or white blood cell count) with age and sex were assessed by 2-way factorial analysis of variance (ANOVA). Differences in the cytokine concentration between the sex and age

‖Address correspondence to Constance R. Chu, MD, Department of Orthopaedic Surgery, Stanford University School of Medicine, 450 Broadway Street, MC 6342, Redwood City, CA 94063, USA (email: chucr@stanford.edu).

*Department of Orthopaedic Surgery, Stanford University School of Medicine, Stanford, California, USA.

†Veterans Affairs Palo Alto Health Care System, Palo Alto, California, USA.

‡Division of Immunology and Rheumatology, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA.

§Division of Hematology, Department of Medicine, Stanford University School of Medicine, Stanford, California, USA.

TABLE 1
Human Subjects and PRP Characteristics[a]

	Male		Female	
	Young (n = 10)	Older (n = 8)	Young (n = 11)	Older (n = 10)
Age, y	22.8 ± 3.2	55.1 ± 4.7	22.9 ± 3.6	52.3 ± 3.3
Body mass index, kg/m^2	23.4 ± 2.8	24.7 ± 2.5	21.6 ± 1.4	24.9 ± 3.0
Whole blood platelet count, 10^3/uL	216.4 ± 32.2	235.9 ± 64.2	219.8 ± 41.8	224.9 ± 35.8
Whole blood white blood cell count, 10^9/L	6.7 ± 2.0	6.1 ± 1.9	6.1 ± 1.3	5.1 ± 0.9
PRP platelet count, 10^3/uL	525.7 ± 352.3	372.8 ± 125.9	336.3 ± 90.5	386.9 ± 101.0
PRP-fold platelet increase	2.5 ± 1.8	1.7 ± 0.9	1.5 ± 0.4	1.8 ± 0.5
High-sensitivity C-reactive protein, mg/L	0.5 ± 0.3	1.8 ± 2.1	1.0 ± 1.3	1.2 ± 0.7
Hemoglobin A1c, %	4.8 ± 0.2	5.1 ± 0.1	5.0 ± 0.1	5.2 ± 0.3

[a]Data are presented as mean ± SD. PRP, platelet-rich plasma.

groups were analyzed by 2-way factorial ANOVA with interaction terms between age and sex. Although the Bio-Plex assay allows for the comprehensive analysis of 27 cytokines, further analysis was performed on selected cytokines of interest, and these proteins were divided into 3 groups: proinflammatory (interleukin–1 beta [IL-1β] and tumor necrosis factor–alpha [TNF-α]), anti-inflammatory (IL-1 receptor antagonist protein [IRAP]), and growth factors (platelet-derived growth factor [PDGF-BB], fibroblast growth factor–basic [FGF-basic], vascular endothelial growth factor [VEGF], TGF-β1, and IGF-1). Pearson correlation coefficients were calculated for platelet counts of whole blood or PRP compared with cytokine or growth factor levels for each age and sex group. To facilitate the comparison of variation patterns between cytokines, a "normalized" value was calculated in which each individual measurement was divided by the group mean to obtain a ratio. A patient who had a lower than average cytokine level would have a ratio <1.0 for that cytokine and >1.0 if he or she had an above average level. This allowed comparisons between cytokines or growth factors whose absolute levels were several orders of magnitude apart. These normalized values were used to calculate ratios between biomarker groups to assess their differences with age and sex between demographic groups using 2-way factorial ANOVA. Furthermore, hierarchical clustering was performed on the results according to similarities among cytokine levels using Cluster 3.0,[16] and the results were visualized in the heat-map format using Java TreeView 1.1.3.[60]

RESULTS

PRP Preparation and Laboratory Data

The mean ages were 22.8 years (range, 18.5-26.8 years) for young male patients, 22.9 years (range, 18.8-27.6 years) for young female patients, 55.1 years (range, 47.0-58.7 years) for older male patients, and 52.3 years (range, 46.8-59.8 years) for older female patients (Table 1). The mean BMI was 23.4 kg/m^2 in young male patients, 21.6 kg/m^2 in young female patients, 24.7 kg/m^2 in older male patients, and 24.9 kg/m^2 in older female patients. Platelet counts in whole blood did not differ by age or sex (P = .234) (Table 1). The

degree of platelet concentration from whole blood to PRP also did not differ significantly by age or sex (P = .313) (Table 1). White blood cells were not observed in any of the PRP samples. Demographic and laboratory data grouped by age and sex showed significance only by age in which the young group had a lower BMI (P = .01) and showed a trend toward lower high-sensitivity C-reactive protein values (P = .09). Interaction effects were not significant.

Correlation Between Platelet Count and PRP Composition

Platelet counts obtained for whole blood samples before PRP preparation did not correlate with any growth factor or cytokine level at the .05 significance level. Platelet counts for PRP correlating with cytokines and growth factors of interest for young female patients were TGF- β1 (β = 0.736, P = .024) and for young male patients were the following: FGF-basic (β = –0.706, P = .023), TNF-α (β = –0.663, P = .037), and IGF-1 (β = 0.732, P = .016). Platelet counts for PRP from older female and older male patients showed no correlation with any of the cytokines or growth factors studied.

Differences in PRP Composition Among Individual Patients

Individual differences in cytokine and growth factor levels were observed within each age and sex group. A color heat-map representation of the distribution of cytokine and growth factors normalized to a Z score is shown in Figure 1. This heat map demonstrates hierarchical clustering and shows grouping of TGF-β1 and IGF-1 distinct from the cytokines. Furthermore, although older male patients consistently demonstrated, on average, higher cytokine levels, substantial intragroup variation can be seen. The 95% CI for cytokines across and within all groups is demonstrated in Table 2. To further illustrate the wide individual variation in cytokine levels, histogram plots and across-group means for selected cytokines and growth factors are demonstrated in Figure 2, showing a typical bell-curve distribution pattern. Within each group shown, there were no

TABLE 2
Age- and Sex-Related Differences in Selected Cytokines and Growth Factors[a]

Biomarker	Overall, Mean (95% CI)	Female		Male		Male to Female		Young to Older	
		Young, Mean (95% CI)	Older, Mean (95% CI)	Young, Mean (95% CI)	Older, Mean (95% CI)	Difference, %	P Value	Difference, %	P Value
IL-1β, pg/mL	8.74 (7.88-9.59)	7.58 (6.41-8.75)	7.82 (6.14-9.50)	9.23 (7.65-10.82)	10.64 (7.81-13.46)	30.6	.008	−7.4	.304
IRAP, pg/mL	257.02 (231.12-282.92)	210.07 (179.68-240.45)	226.12 (188.27-263.96)	276.24 (225.64-326.85)	327.91 (245.21-410.62)	40.3	<.001	−10.9	.137
TNF-α, pg/mL	120.78 (109.21-132.35)	105.15 (89.51-120.79)	115.84 (86.41-145.28)	122.91 (102.41-143.41)	143.56 (108.00-179.11)	23.1	.048	−12.0	.167
FGF-basic, pg/mL	215.43 (197.79-233.06)	196.80 (164.96-228.64)	191.21 (153.69-228.73)	232.54 (199.94-265.14)	245.44 (193.95-296.93)	24.7	.010	0.1	.827
PDGF-BB, pg/mL	2928.58 (2522.50-3334.66)	2775.86 (1867.39-3684.33)	2382.78 (1860.61-2904.95)	3364.96 (2513.39-4216.53)	3201.70 (1848.13-4555.28)	24.3	.087	10.9	.491
VEGF, pg/mL	125.04 (105.97-144.12)	119.87 (72.04-167.71)	97.07 (75.59-118.55)	132.68 (105.85-159.51)	155.97 (84.69-227.26)	33.8	.060	2.6	.989
TGF-β1, ng/mL	106.18 (97.21-115.14)	87.92 (76.40-99.43)	97.70 (79.86-115.53)	115.11 (98.43-131.79)	123.99 (98.12-149.85)	21.9	.002	−7.0	.248
IGF-1, ng/mL	76.93 (67.22-86.63)	97.04 (79.16-114.93)	53.66 (42.03-65.29)	98.19 (81.31-115.07)	54.15 (43.96-64.34)	6.8	.903	44.8	.000

[a]FGF-basic, fibroblast growth factor–basic; IGF-1, insulin-like growth factor–1; IL-1β, interleukin–1 beta; IRAP, IL-1 receptor antagonist protein; PDGF-BB, platelet-derived growth factor; TGF-β1, transforming growth factor–beta 1; TNF-α, tumor necrosis factor–alpha; VEGF, vascular endothelial growth factor.
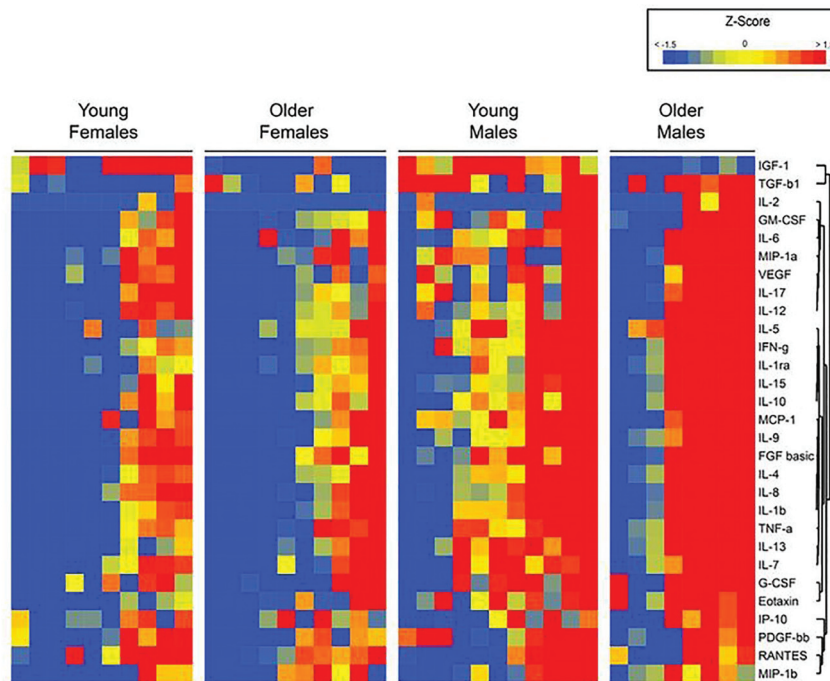

Figure 1. Heat-map comparison of cytokines and growth factors. This map demonstrates within- and across-group variations in cytokine and growth factor levels. Each individual molecule is normalized to the mean of the entire group and the color determined by the corresponding *Z* score, with low scores represented in blue and high scores represented in red. Note that there are patients with low (blue) and high (red) levels of cytokines in each group.

differences in subdivisions (eg, between young female and older female patients), and no interaction effects between age and sex were observed.

Differences in PRP Composition Between Age and Sex Groups

Significant differences in PRP between male and female patients were observed in content for the majority of the cytokines studied (Figure 1) as well as the cytokines and

growth factors selected for further analysis (Figure 2). For these proteins, differences between female and male patients were observed for the following proinflammatory cytokines: IL-1β (7.71 vs 9.83 pg/mL, respectively; *P* = .008) and TNF-α (110.5 vs 131.6 pg/mL, respectively; *P* = .048); for the anti-inflammatory cytokine IRAP (218.0 vs 298.0 pg/mL, respectively; *P* < .001); and for the following growth factors: FGF-basic (194.0 vs 237.9 pg/mL, respectively; *P* = .010) and TGF-β1 (92.8 vs 118.8 ng/mL, respectively; *P* = .002). There was a trend toward lower concentrations in female than male patients for PDGF-
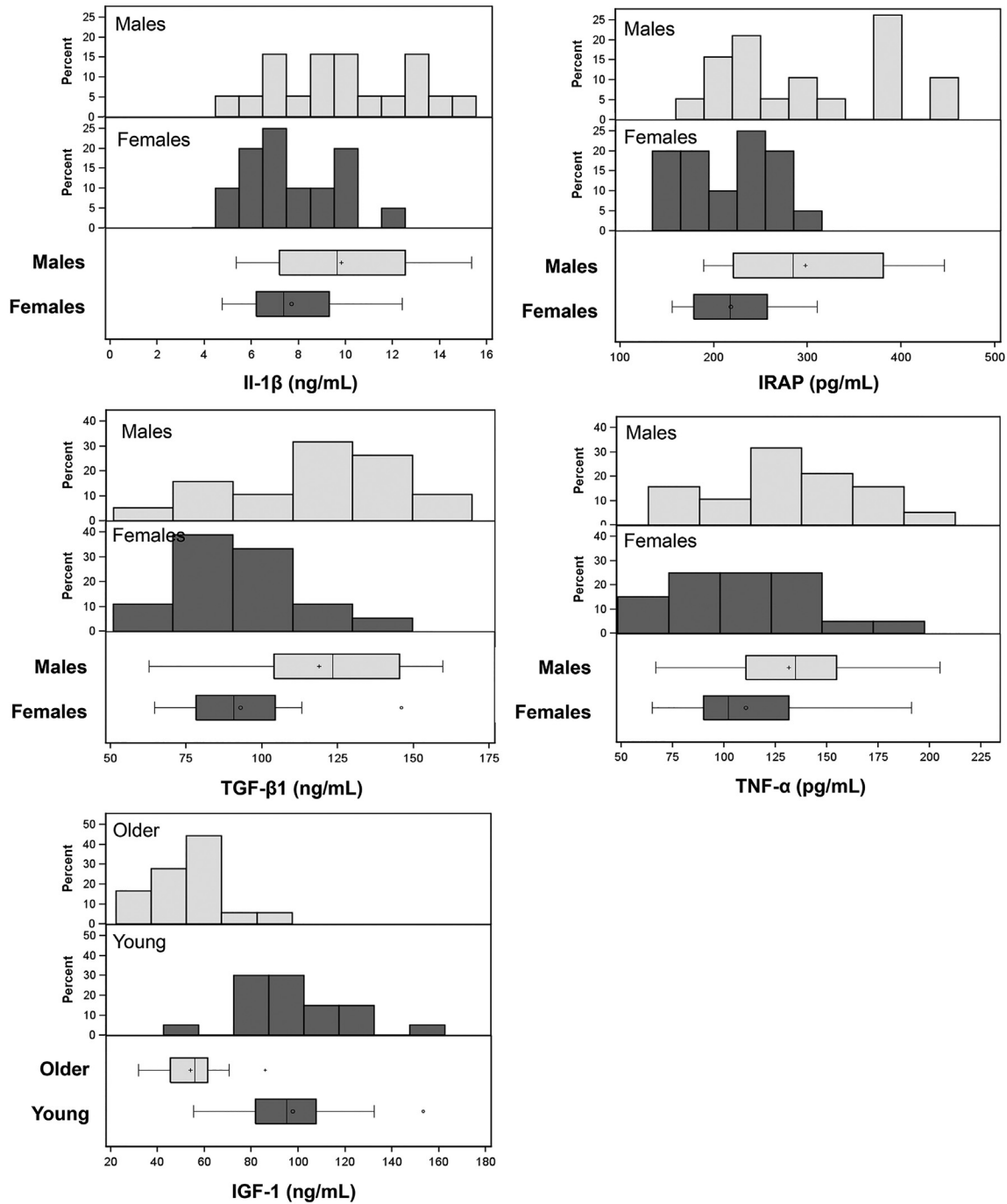
Figure 2. Cytokine and growth factor distributions of female compared with male patients. Histogram plots grouped by sex for interleukin–1 beta (IL-1β) (7.71 vs 9.83 pg/mL, respectively; $P = .008$), tumor necrosis factor–alpha (TNF-α) (110.5 vs 131.6 pg/mL, respectively; $P = .048$), IL-1 receptor antagonist protein (IRAP) (218.0 vs 298.0 pg/mL, respectively; $P < .001$), and transforming growth factor–beta 1 (TGF-β1) (92.8 vs 118.8 ng/mL, respectively; $P = .002$) as well as insulin-like growth factor–1 (IGF-1) grouped by age (97.6 vs 53.8 ng/mL, for younger vs older; $P < .001$). Although an overall pattern of higher cytokine or growth factors is apparent in male compared with female patients, there is intragroup variation and overlap in the distributions.

BB (2579.3 vs 3296.2 pg/mL, respectively; $P = .087$) and VEGF (108.4 vs 142.4 pg/mL, respectively; $P = .060$), while IGF-1 did not show significant differences ($P = .903$) between male and female patients (Table 2). Two-way

ANOVA did not show significant differences between the young and older age groups for any of these cytokines and growth factors except for IGF-1 (97.6 vs 53.8 ng/mL, respectively; $P < .001$) (Table 2). The percentage increase
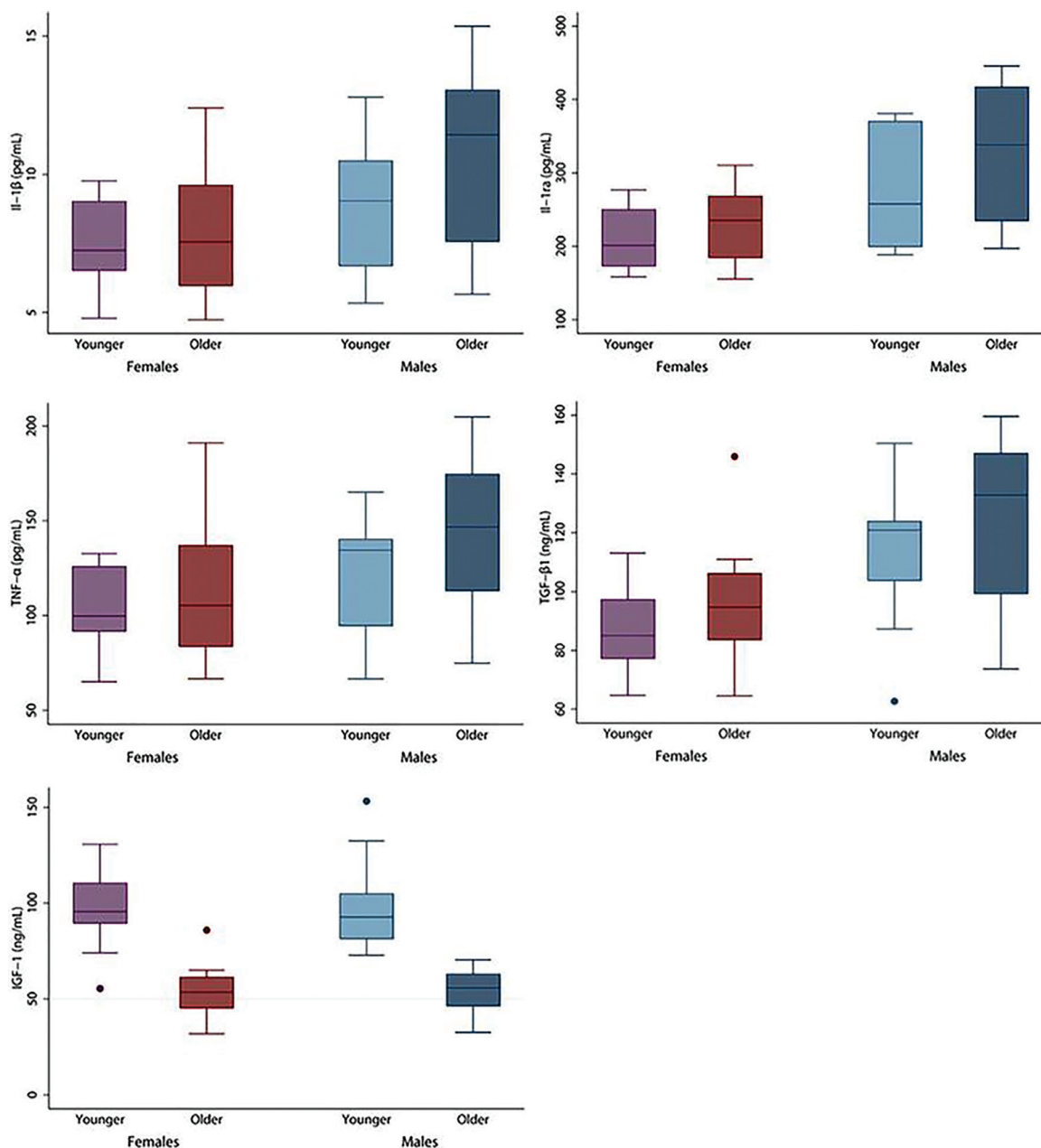
Figure 3. Cytokine and growth factor distributions of female compared with male patients by age group. Box plots are grouped visually by age and sex, with significant differences between females and males in: interleukin-1 beta (IL-1β) (7.71 vs 9.83 pg/mL, respectively; $P = .008$), tumor necrosis factor-alpha (TNF-α) (110.5 vs 131.6 pg/mL, respectively; $P = .048$), IL-1 receptor antagonist protein (IRAP) (218.0 vs 298.0 pg/mL, respectively; $P < .001$), transforming growth factor-beta 1 (TGF-β1) (92.8 vs 118.8 ng/mL, respectively; $P = .002$). Insulin-like growth factor-1 (IGF-1) showed significant differences between younger and older groups (97.6 vs 53.8 ng/mL, respectively; $P < .001$). Boxes demonstrate the median, 25th percentile, and 75th percentile. Upper and lower adjacent lines demonstrate upper and lower adjacent values, respectively. Outside values are shown by dots representing discrete data points.

in male compared with female patients for each biomarker that showed significant differences at the 5% alpha level were the following, in descending order: IRAP (40.3%), VEGF (33.8%), IL-1β (30.6%), FGF-basic (24.7%), PDGF-BB (24.3%), TNF-α (23.1%), and TGF-β1 (21.9%) (Table 2).

Young patients had a 44.8% greater mean IGF-1 level than older patients. There were no significant correlations between high-sensitivity C-reactive protein and cytokine levels. Furthermore, the interaction between age and sex was not significant for any of the cytokines (Figures 1 and 3).
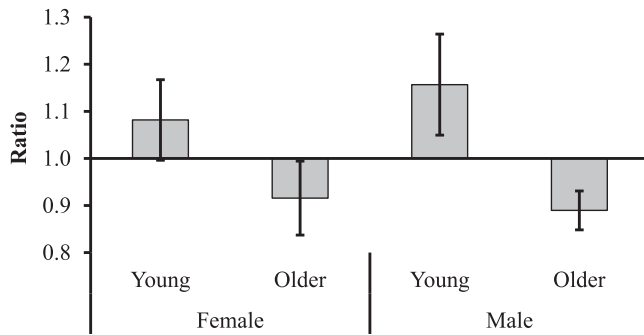
Figure 4. Ratio of growth factors to proinflammatory cytokines. The ratio of normalized values of growth factors (platelet-derived growth factor [PDGF-BB], fibroblast growth factor–basic [FGF-basic], vascular endothelial growth factor [VEGF], transforming growth factor–beta 1 [TGF-β1], and insulin-like growth factor–1 [IGF-1]) to proinflammatory (interleukin–1 beta [IL-1β] and tumor necrosis factor–alpha [TNF-α]) cytokines was higher in the young group compared with the older group (1.12 vs 0.90, respectively; *P* = .018) but not significantly different between male and female patients (1.04 vs 0.99, respectively; *P* = .78).

Ratios Between Proinflammatory Mediators and Anti-inflammatory Mediators or Growth Factors

The ratio of anti-inflammatory (IRAP) and proinflammatory (IL-1β) cytokines was higher in male compared with female patients (1.036 vs 0.977, respectively; *P* = .051) (Figure 3), and there was no age-related difference (0.987 young vs 1.027 older, respectively; *P* = .28). The ratio of growth factors (PDGF-BB, FGF-basic, VEGF, TGF-β1, and IGF-1) to proinflammatory (IL-1β and TNF-α) cytokines was higher in the young group compared with the older group (1.12 vs 0.90, respectively; *P* = .018) (Figure 4) but not significantly different between male and female patients (1.04 vs 0.99, respectively; *P* = .78).

DISCUSSION

The results of this study show significant differences in the composition of PRP between healthy male and female patients. Specifically, PRP from male patients contained a higher level of cytokines such as IL-1β, IRAP, and TNF-α and growth factors such as PDGF-BB, VEGF, and TGF-β1 compared with female patients. Age-related differences were less evident, with PRP from older patients showing a lower IGF-1 content. Furthermore, substantial within-group variability in the PRP composition was observed. The clinical laboratory test of high-sensitivity C-reactive protein did not correlate with PRP cytokine levels, suggesting that it is of low utility as a surrogate marker for levels of inflammatory mediators in PRP.

While some previous studies have shown differences between PRP prepared using different systems[11,17,49,66] and other reports have suggested that individual differences in the PRP composition may contribute to variability in the

PRP composition,[7,25] prior studies typically have reported mean PRP values found in the study cohort.[72] Thus, information on differences in the PRP composition between different people and different groups of people is lacking. This study specifically evaluated between-person differences in the PRP composition using a standard hematology protocol to generate leukocyte-poor PRP.[45] The data further highlight that variability in the composition of PRP is high and that this variability complicates determining the mechanisms of action and clinical efficacy of PRP.

Despite the significant intergroup differences in the PRP composition, there was also significant intragroup variation among individual patients. These findings have high potential relevance to the clinical use of PRP. Unlike the administration of a medication or substance with known concentrations of an active ingredient or bioactive factor, there are no proteomic standards for PRP, which is minimally processed from whole blood. In addition to the previously shown variability in PRP depending on processing techniques and diurnal variations, we have further shown that sex appears to influence the composition of PRP in healthy patients. As PRP variability cannot be precisely controlled because of the minimally manipulated nature of the treatment, this study supports accounting for the composition of PRP in future basic and clinical studies of tissue and clinical effects.

As there are an exceptionally high number of variables related to PRP treatment, it would not be feasible to include every potential factor for evaluation. Thus, it would be important for a consensus panel of experts to develop basic guidelines based on the best available current literature. Platelet and white blood cell counts of the PRP prepared for administration as well as factors considered relevant to the disease being treated may be an appropriate reference point.

In this study, factors were selected for evaluation based on an interest in assessing PRP injections for the treatment of osteoarthritis in which leukocyte-poor preparations may reduce concerns for excessive proinflammatory activity.[8] In addition to platelet and white blood cell levels, special emphasis was placed on studying the inflammatory mediators of IL-1β and TNF-α, the anti-inflammatory protein IRAP, and the cartilage anabolic growth factors TGF-β1 and IGF-1. The growth factors PDGF-BB, VEGF, and FGF-basic were additionally included because of their prominence in PRP and importance to tissue repair.

For example, the proinflammatory cytokine IL-1β is a well-known instigator of cartilage damage and osteoarthritis and is used experimentally to induce an osteoarthritic state in animal models.[77] In conjunction with other inflammatory markers such as IL-1β,[27] TNF-α both potentiates cartilage damage and the development of osteophytes as well as prevents the migration of chondroprogenitors for cartilage healing[5] and has been shown to play a role in potentiating pain responses.[32,42,48] In this study, both of these cytokines showed significantly higher levels in male compared with female patients.

However, the observation of higher overall cytokine levels in male compared with female patients cannot be interpreted in isolation, as the relationships are complex. In

this study, IRAP levels were also significantly higher in male than female patients. IRAP is a competitive antagonist to the proinflammatory effects of IL-1 that works by binding to the IL-1 receptor.[52,75,78] This is just one example of the checks and balances within the cytokine milieu that further complicates the attribution of PRP effects to one or a few factors.

The motivation behind the widespread clinical use of PRP includes the potential role of PDGFs to promote healing in musculoskeletal injuries. This study showed that TGF-β1 had significantly higher levels in the PRP of male patients compared with female patients, while the growth factor IGF-1 had significantly higher levels in the PRP of young patients compared with older ones. It has been shown that TGF-β1 promotes chondrocyte differentiation and cartilage health[3,24,40,71] but may contribute to pathological conditions such as tendon healing[15] and bone changes of osteoarthritis.[18,76] Thus, the context and disease state for which PRP treatment is intended would necessarily affect desired levels of a particular growth factor.

For example, IGF-1 also shows positive effects on cartilage health and metabolism,[6,14,26,50,68,79] particularly in the hypoxic, dynamic loading environment specific to chondrocytes.[36,73] In addition, IGF-1 has shown chondroprotective effects against inflammatory damage from cytokines such as IL-1β.[43] However, diseased cartilage shows reduced sensitivity to the anabolic effects of IGF-1.[13] This study demonstrated lower IGF-1 levels in older patients. Given the decreased sensitivity of aged, arthritic chondrocytes to IGF-1–mediated chondrogenesis,[33,69] the reduction in IGF-1 levels with age further raises questions concerning whether this protein is relevant for the treatment of osteoarthritis, which is more commonly observed in older patients. This example illustrates the complexity in relating proteomic concentrations in PRP to tissue effects. Further research on the effect of age, sex, osteoarthritic disease state, and patient health on PRP composition and treatment effects is needed.

This study showing the wide variation in PRP composition and patterns of PRP composition based on age and sex emphasizes the importance of a personalized approach in which the potential effects of PRP variation on tissue and clinical outcomes are accounted for. Despite the high number of variables and the complexity of evaluating PRP actions, the data from our study suggest that there may be either demographic or proteomic markers of likely responders and nonresponders to PRP treatment and that further study evaluating these markers against clinical outcomes is needed. While age alone did not readily account for proteomic differences in healthy patients, the ratio of growth factors to inflammatory cytokines was positive in the young group and negative in the older group and potentially suggestive of a higher regenerative capacity in the young. These ratios, while theoretical, illustrate the importance of pursuing a broader understanding of the numerous factors in PRP by evaluating the balance between anabolic and catabolic factors. Further exploration of these relationships is needed.

This work highlights another important area of sex-related differences in biology and the healing potential

that deserves further study. Hormonal differences drive many sex-related differences between men and women. While several studies have shown an increase in inflammatory markers with menopause, this was not observed in our perimenopausal group of older female patients.[54,62] The data from our study showing proteomic differences in PRP provide additional insight into the biological differences between the sexes and raise the question as to whether male and female patients may respond differently to PRP treatment. Male and female patients have been previously shown to demonstrate differences in innate healing responses, another component in the response to PRP treatment. Male patients and specimens have shown improved healing of mucosal tissue in human models[19] as well as bone regeneration[65] and ligament reconstruction[28] in animal models. In addition to healing and regeneration, a potential analgesic effect of PRP may be also affected by sex-related factors such as the findings that women with osteoarthritis tend to exhibit more widespread pain and greater sensitivity to mechanical stimuli than men[4] and tend to rate clinical pain higher for musculoskeletal conditions such as sprains and arthropathies.[58]

In addition to inherent differences among individual patients in the PRP composition, host factors may also affect responses to PRP treatment. For example, our study demonstrated that platelet counts for PRP from older female and older male patients showed no correlation with any of the cytokines or growth factors studied. This may relate to reduced platelet competency or a lower platelet activation potential in older patients. Host differences such as sex may indicate not only different cytokine levels, as demonstrated in this study, but also varying responses to these cytokines. Women tend to mount a more aggressive immunological response to inflammation and faster recovery of the preinflammatory state,[10] and this may result in lower baseline cytokine levels. Lower cytokine concentrations in PRP, therefore, may prompt an equivalent response in women as it does in men. Furthermore, host age may affect the potential for regenerative responses. As previously mentioned, older, arthritic chondrocytes show a blunted response to IGF-1.[33,69]

Additionally, host behaviors such as diet and exercise may also affect responses to PRP content. One study demonstrated that dynamic compression mimicking exercise with C-type natriuretic peptide administration mitigated inflammatory chondrocyte damage by IL-1β[55] and that male participants tend to exhibit greater decreases in inflammatory markers such as C-reactive protein in response to exercise.[74] A recent study by Nazli et al[47] showed decreased IGF-1–mediated proteoglycan production in mice on a high-fat diet compared with mice on a low-fat diet. Medications may also play a role. For example, dexamethasone was shown to be synergistic with increased IGF-1 levels in preventing cytokine-mediated chondrocyte damage in an in vitro model.[31] This leads to a complex interplay of PRP composition and host factors, underscoring the importance of individual consideration in PRP clinical use.

Limitations of this study include that PRP was obtained only from healthy patients. As such, the findings may be

more reflective of the PRP composition in those receiving treatment for athletic injuries, who are more likely to be healthy, than for those seeking relief of knee osteoarthritis, who may be older and have comorbidities such as obesity, diabetes, or heart conditions. Although this study standardized whole blood collection time to a single morning hour, there may also have been diurnal variations in personal habit that may have made this uniform collection time relatively early or late for different individual patients. Furthermore, this study utilized a previously described freeze-thaw method of obtaining platelet releasate for analysis,[57,67] which may not reflect in vivo release kinetics in which differences in the "platelet activation potential" between individual patients may be another source of variability. The freeze-thaw method to obtain PRP releasate was chosen because activation with standard "activators" such as $CaCl_2$ or thrombin results in fibrinogen cleavage and clot formation that is undesirable for clinical applications, such as intra-articular injections for knee osteoarthritis, and would also sequestrate growth factors and proteins in the clot.[12,59] A single freeze-thaw cycle has long been used for this purpose.[9] Recently, a study examining pooled platelet lysates has shown that multiple freeze-thaw cycles have differing effects on the concentrations of various growth factors and anti-inflammatory mediators, which highlights another potential source of variability.[64] Finally, we used a standard PRP preparation protocol as defined in hematology[45] as opposed to a commercial system. The results from this study were not intended to provide a standard or reference for commercial systems, for clinical treatment, or to model clinical platelet release kinetics and should not be interpreted in this fashion. Rather, the purpose of this study was to show differences between PRP from different individual patients and groups of patients processed and analyzed using the same techniques.

In summary, this study shows significant variation of PRP among individual patients and that this variation is influenced by age and sex. Combined with the known variability between PRP generated by different systems, these data highlight that future research needs to account for the composition of PRP used. Host factors may also affect responses to PRP treatment. Given the multitude of factors impacting PRP treatment, substantial additional basic and clinical research is needed to determine the mechanisms of action of PRP on different pathological processes, which components support or contradict these mechanisms, and whether there are groups or categories of patients that are more or less likely to benefit from PRP. Ultimately, it is likely that a personalized approach to the clinical use of PRP will be needed.

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