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Effect of Leukocyte Concentration on the Efficacy of Platelet-Rich Plasma in the Treatment of Knee Osteoarthritis

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Background: Leukocyte-poor platelet-rich plasma (LP-PRP) is hypothesized to be more suitable for intra-articular injection than leukocyte-rich PRP (LR-PRP) in the treatment of knee osteoarthritis.

Purpose: To compare clinical outcomes and rates of adverse reactions between LP-PRP and LR-PRP for this application.

Study Design: Meta-analysis.

Methods: The MEDLINE, EMBASE, and Cochrane databases were reviewed. The primary outcome was the incidence of local adverse reactions. Secondary outcomes were the changes in International Knee Documentation Committee (IKDC) subjective score and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score between baseline and final follow-up measurements. A Bayesian network meta-analysis was performed, with a post hoc meta-regression to correct for baseline differences in WOMAC scores. Treatment rankings were based on surface under the cumulative ranking (SUCRA) probabilities.

Results: Included in the analysis were 6 randomized controlled trials (evidence level 1) and 3 prospective comparative studies (evidence level 2) with a total of 1055 patients. Injection of LP-PRP resulted in significantly better WOMAC scores than did injection of hyaluronic acid (mean difference, –21.14; 95% CI, –39.63 to –2.65) or placebo (mean difference, –17.84; 95% CI, –34.95 to –0.73). No such difference was observed with LR-PRP (mean difference, –14.28; 95% CI, –44.80 to 16.25). All treatment groups resulted in equivalent IKDC subjective scores. The SUCRA analysis showed that LP-PRP was the highest ranked treatment for both measures of clinical efficacy (WOMAC and IKDC). Finally, PRP injections resulted in a higher incidence of adverse reactions than hyaluronic acid (odds ratio, 5.63; 95% CI, 1.38-22.90), but there was no difference between LR-PRP and LP-PRP (odds ratio, 0.78; 95% CI, 0.05-11.93). These reactions were nearly always local swelling and pain, with a single study reporting medical side effects including syncope, dizziness, headache, gastritis, and tachycardia (17/1055 total patients).

Conclusion: LP-PRP results in improved functional outcome scores compared with hyaluronic acid and placebo when used for treatment of knee osteoarthritis. LP-PRP and LR-PRP have similar safety profiles, although both induce more transient reactions than does hyaluronic acid. Adverse reactions to PRP may not be directly related to leukocyte concentration.

Keywords: platelet-rich plasma; knee osteoarthritis; injection; leukocyte; white blood cell

Platelet-rich plasma (PRP) is a concentrate derived from peripheral blood, and it is now frequently used in sports medicine and orthopaedics. It is hypothesized to have regenerative, anti-inflammatory, analgesic, and antimicrobial properties.[30] As a result, PRP has been studied as an adjunct for bone healing,[2,46,47] cartilage healing,[§] and chronic tendinopathy[16,37] and in the setting of surgical procedures such as rotator cuff repair,[7] Achilles tendon repair,[42] and anterior cruciate ligament reconstruction.[28,33,38]

An area of particular interest has been the treatment of knee osteoarthritis (OA).[30] In vitro and ex vivo studies have provided the foundation for this interest, and positive effects of PRP have been observed, including chondrogenic

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§References 6, 12, 13, 15, 17, 25, 36, 40, 41, 44.

differentiation of pluripotent mesenchymal cells with expression of cartilage specific genes, chondrocyte proliferation, increased extracellular matrix production, and inhibition of catabolic pathways.[14,27,34,35,43,45] As a result, multiple clinical trials have been performed assessing the efficacy of PRP in the treatment of knee OA. Although in vitro and ex vivo data are promising, clinical study design and subsequent results have varied significantly.[§] Qualitative reviews and 2 meta-analyses, while promising, failed to provide conclusive evidence of the efficacy of PRP for this indication.[9,20,23] In fact, the American Academy of Orthopaedic Surgeons Clinical Practice Guidelines consortium concluded that they "could not recommend for or against PRP in the treatment of symptomatic knee osteoarthritis."[22(p1886)]

In the face of these inconsistent results, there has been growing interest in characterizing the cellular composition of the various commercially available PRP preparations, in an effort to identify the ideal PRP contents.[4,5,11] Special attention has been devoted to the leukocyte (white blood cell [WBC]) concentration in PRP. High concentrations of WBC have been shown to increase the expression of catabolic cascades and inflammatory markers such as interleukin-1 and tumor necrosis factor–alpha.[29,31] In cultured synoviocytes, leukocyte-rich PRP (LR-PRP) causes cell death and the expression of multiple inflammatory markers.[4] Similar results were found in vivo in a rabbit tendon model.[11] In addition, a prospective comparative study provided early evidence that painful reactions are more common with LR-PRP.[13] On the basis of this evidence, it has been suggested that leukocyte-poor PRP (LP-PRP) would be most suitable for intra-articular injection[3,31]; however, there is clearly a paucity of clinical evidence to substantiate this recommendation.

The goal of the present study was to provide a quantitative synthesis of the clinical data comparing LP-PRP and LR-PRP in the treatment of knee OA. Despite the availability of several randomized controlled trials (RCTs), LR-PRP and LP-PRP were directly compared in only a single trial,[13] while they were each compared with common references (hyaluronic acid [HA] or placebo) in multiple trials.[¶] With use of network meta-analysis (NMA) techniques, information beyond that available with a traditional meta-analysis can be obtained by comparing multiple treatments for the same clinical condition.[19] Specifically, NMA allows for the combination of direct and indirect evidence for specific pairwise comparisons, providing a robust estimate of the true treatment effect, even if some treatments have never been directly compared in an RCT.[19] This method is gaining popularity in health decision research[19] and is currently used by the American Academy of Orthopaedic Surgeons to prepare clinical practice guidelines.[32] Our hypothesis was that LP-PRP would result in fewer local adverse reactions and lead to improved functional outcome scores compared with LR-PRP in an NMA model.

[¶]References 6, 12, 13, 17, 25, 36, 40, 41, 44.

METHODS

Study Design and Data Collection

The guidelines from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; www.prisma-statement.org) were used to design our review of the literature. The MEDLINE, EMBASE, and Cochrane databases were reviewed for all English-language studies published before August 2014. We used the iterative "breadth-first" search approach, which was specifically designed for populating NMA models due to its ability to identify indirect comparison studies that do not appear in an initial search.[18] The specific key phrases were as follows: (1) platelet rich plasma knee osteoarthritis, (2) platelet rich plasma leukocyte knee osteoarthritis, (3) platelet rich plasma hyaluronic acid knee osteoarthritis, (4) platelet rich plasma placebo knee osteoarthritis. Eligibility criteria included (1) an evidence level 1 or 2 randomized design; (2) a study design comparing LR-PRP or LP-PRP to a control treatment, or a direct comparison of LR-PRP and LP-PRP; and (3) full reporting of outcomes and use of appropriate statistical methods. Exclusion criteria included (1) studies that were not available in English, (2) unpublished studies, and (3) randomized trials comparing variables beyond the scope of this review (eg, number of PRP injections). All abstracts were reviewed in duplicate by 2 of the authors (J.C.R., B.M.S.) and were assessed based on the above criteria. The full text of eligible studies was then reviewed by the same authors to determine final inclusion. Data were then extracted in duplicate from all studies using a standardized form created by the authors at the onset of the review. Inconsistencies between reviewers were resolved by joint review and consensus opinion.

Definition of Leukocyte-Rich and Leukocyte-Poor PRP

The methods section of each included study was carefully reviewed for descriptions of the leukocyte concentration in the final PRP product used for intra-articular injection. When insufficient information was provided, study authors were contacted, and in all cases responses were obtained. If the study authors did not record leukocyte concentration, the manufacturer documentation for the PRP system that they used was reviewed to extract detailed information about leukocyte concentration. LR-PRP was defined as PRP having a WBC concentration greater than 100% that of whole blood. Conversely, LP-PRP was defined as PRP having a WBC concentration less than 100% of whole blood. With these methods, all preparations could be unambiguously assigned to the LR or LP categories.

Data Synthesis and Statistical Analysis

All outcome variables reported in the literature were included in the prespecified data extraction sheet. However, only variables reported in both LR-PRP and LP-PRP study arms were included in the NMA. For continuous outcomes, the summary measure was the difference of means. For dichotomous outcomes, the summary

TABLE 1
Summary of Included Studies[a]

Study (Year)	Level of Evidence	Study Design	Treatment 1	Treatment 2	n (Treatment 1)	n (Treatment 2)
Cerza et al (2012)[6]	1	RCT	HA	LP-PRP	60	60
Filardo et al (2012)[12]	1	RCT	HA	LR-PRP	55	54
Hart et al (2013)[17]	1	RCT	Placebo	LP-PRP	50	50
Patel et al (2013)[36]	1	RCT	Placebo	LP-PRP	23	51
Sanchez et al (2012)[40]	1	RCT	HA	LP-PRP	74	74
Spakova et al (2012)[44]	1	RCT	HA	LR-PRP	60	60
Filardo et al (2012)[13]	2	PCS	LR-PRP	LP-PRP	72	72
Kon et al (2011)[25]	2	PCS	HA	LR-PRP	100	50
Say et al (2013)[41]	2	PCS	HA	LP-PRP	45	45

[a]HA, hyaluronic acid; LP-PRP, leukocyte-poor platelet-rich plasma; LR-PRP, leukocyte-rich platelet-rich plasma; PCS, prospective comparative study; RCT, randomized controlled trial.

measure was the odds ratio. Intention-to-treat datasets were used whenever available. The primary outcome was the incidence of local adverse reactions to the intra-articular knee injections. Secondary outcomes were the changes in International Knee Documentation Committee subjective score (ΔIKDC) and Western Ontario and McMaster Universities Osteoarthritis Index score (ΔWOMAC) between baseline and final follow-up measurements.

Before NMA is performed, it is essential to verify that the assumption of transitivity is valid.[21] Briefly, transitivity means that the results of direct and indirect evidence are consistent. For example, if treatment C is more efficacious than treatment B, and B is more efficacious than A, then C must be more efficacious than A.[21] The 2 validated methods for assessing the assumption of transitivity are (1) comparison of the distribution of effect modifiers between studies and (2) calculating the difference between direct and indirect evidence in all pairwise comparisons within the network.[21,26] For this study, all available demographic data for each of the treatment groups were compiled. Continuous variables were summarized with frequency weighted means (with the weight being proportional to the number of study participants) to account for the variability in study size. Dichotomous variables were reported as cumulative frequencies. These data were compared between groups using analysis of variance (ANOVA) for continuous variables and chi-square contingency analysis for dichotomous outcomes. The difference between direct and indirect estimates for all possible treatment comparisons was then measured. The significance of these differences was calculated using the *network* package in Stata (Stata Corp).[21,26]

An NMA model was fitted into a Bayesian context, which properly accounts for correlations between effect sizes from multiarm studies and maintains within-study randomization.[8] The model was built using the *mvmeta* command in Stata, as previously described.[8] A random-effects model was used to provide a more conservative estimate of effect sizes, and a common heterogeneity was assumed across comparisons. Effect sizes were reported with 95% credible intervals (95% CrI) using the *intervalplot* command in Stata, to provide a forest plot for all possible pairwise treatment comparisons.[8] Given the asymmetric distribution of baseline WOMAC scores between groups, a meta-regression was performed on the WOMAC data to assess whether observed differences were driven by an underlying difference in study populations. Meta-regression is an extension of standard meta-analysis that investigates the extent to which statistical heterogeneity can be related to one or more study characteristics.[19] Meta-regression was performed by use of the *metareg* command in Stata, which includes the Knapp-Hartung modification to minimize the false-positive rate, as recommended.[24]

To rank the treatments based on individual outcome variables, the surface under the cumulative ranking (SUCRA) probabilities, as well as the probability of each treatment being the best, were reported.[39] SUCRAs are expressed as percentages and compare each intervention to a hypothetical intervention that is always the best without uncertainty.[39] A SUCRA of $x\%$ means that the treatment achieves x percent of the effectiveness of the imaginary treatment; therefore, a larger SUCRA is associated with a more effective treatment. SUCRA is preferred to ranking treatments based solely on their probability of being the best, since the latter can give spuriously high ranks to treatments for which little evidence is available.[8] As a result, final rankings were based on SUCRA values.

All statistical analyses were performed in Stata 13 using the *mvmeta* package for NMA and the *network graphs* package for graphical representation of results (http://www.mtm.uoi.gr).

RESULTS

Evidence Base

Included in the analysis were 6 RCTs (evidence level 1) and 3 prospective comparative studies (evidence level 2) published between 2011 and 2013 and including a total of 1055 patients. Details of the literature search are shown in a PRISMA flowchart (Figure 1). A network diagram summarizing the available data is presented in Figure 2. The included studies are summarized in Table 1. Four different treatment options were identified in the included

TABLE 2
Leukocyte Concentration of Platelet-Rich Plasma Preparations Used in the Included Studies[a]

Study Cohort	PRP Characteristics
Leukocyte-rich formulations	
Filardo et al (2012)[12]	WBC concentration increased 120% in PRP compared with whole blood.
Spakova et al (2012)[44]	WBC concentration increased 360% in PRP compared with whole blood.
Kon et al (2011)[25]	Authors contacted: WBC concentration not recorded; however, no WBC reduction was performed, and authors qualitatively described their PRP as leukocyte rich.
Filardo et al (2012)[13]	WBC concentration increased 140% in PRP compared with whole blood.
Leukocyte-poor formulations	
Patel et al (2013)[36]	Total leukocyte count of 0 in PRP.
Hart et al (2013)[17]	WBC concentration decreased 50% in PRP compared with whole blood.
Sanchez et al (2012)[40]	Authors contacted: PRGF-Endoret preparation used. Manufacturer details reviewed; preparation is reported to have little to no WBC content.
Cerza et al (2012)[6]	Authors contacted: Arthrex Autologous Conditioned Plasma (ACP) used. Manufacturer details reviewed; preparation reduces WBC content by at least 85% when compared with whole blood.
Filardo et al (2012)[13]	Total leukocyte count of 0 in PRP.
Say et al (2013)[41]	Authors contacted: PRGF-Endoret preparation used. Manufacturer details reviewed; preparation reported to have little to no WBC content.

[a]PRGF, plasma rich in growth factors; PRP, platelet-rich plasma; WBC, white blood cell.

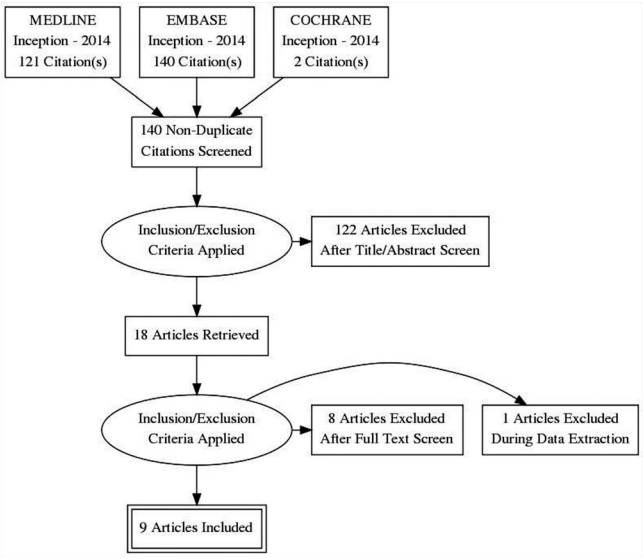

Figure 1. PRISMA study flowchart.

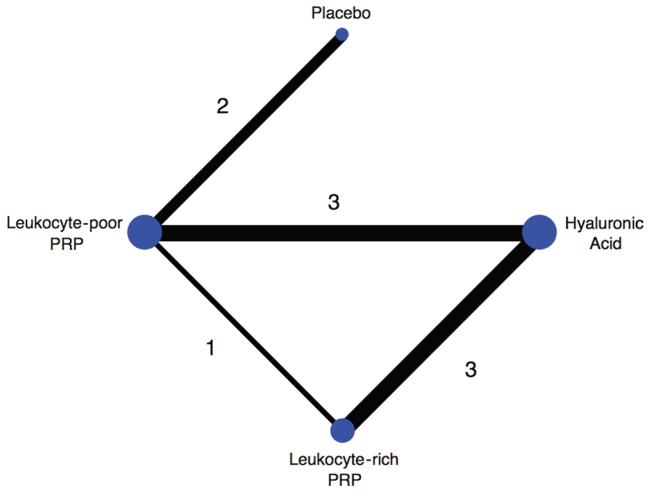

Figure 2. Network plot of included studies. The network plot represents the evidence available for building a network meta-analysis model. Each node represents a treatment option, and an edge connecting 2 nodes indicates that they have been directly compared in a study. Nodes are weighted according to the number of studies including the respective interventions. Edges are weighted and labeled based on the number of studies comparing the 2 treatments connected by the edge. PRP, platelet-rich plasma.

literature: (1) placebo injections, including normal saline and/or local anesthetic; (2) HA; (3) LR-PRP; and (4) LP-PRP. Table 2 presents the leukocyte concentration of each PRP preparation included in the study.

While a total of 12 outcome variables were reported (WOMAC, IKDC subjective, IKDC objective, Tegner, Marx, Knee Injury Osteoarthritis Outcome Score, visual analog scale, EuroQol visual analog scale, Lequesne index, magnetic resonance appearance, 36-Item Short Form Health Survey, and number of adverse reactions), only 3 variables were reported in a sufficient number of studies to be included in the final analysis: (1) WOMAC score (4 studies), (2) IKDC subjective score (4 studies), and (3) number of adverse reactions (5 studies).

To assess the assumption of transitivity, a comparison of baseline characteristics between treatment groups was performed, and the results are presented in Table 3. Overall, the treatment groups were homogeneous. There were no significant differences in age, sex distribution, body mass index, and number of PRP injections. ANOVA of baseline WOMAC and IKDC scores was not possible due to an insufficient number of studies reporting these variables. As expected, LR-PRP had higher platelet and WBC

TABLE 3
Patient Demographics Based Stratified by Treatment[a]

	Placebo	Hyaluronic Acid	Leukocyte-Rich PRP	Leukocyte-Poor PRP	*P* Value
Age					.16
Study arms (patients), n	2 (73)	6 (394)	4 (236)	6 (352)	
Mean ± SD, y	56.8 ± 2.2	57.4 ± 4.2	52.1 ± 1.9	57.9 ± 4.8	
% Male					.36
Study arms (patients), n	2 (73)	6 (394)	4 (236)	6 (352)	
Mean ± SD	41.9 ± 14.9	47.1 ± 12.6	60.5 ± 4.5	47.5 ± 17.9	
Body mass index					.76
Study arms (patients), n	2 (73)	5 (334)	4 (236)	5 (292)	
Mean ± SD, kg/m^2	27.3 ± 0.7	27.4 ± 2.4	26.2 ± 1.3	27.6 ± 2.4	
PRP injections					.69
Study arms (patients), n	NA	NA	4 (236)	6 (352)	
Mean ± SD, n	—	—	3 ± 0	3.5 ± 2.4	
Platelet count					.01
Study arms (patients), n	NA	NA	2 (132)	3 (173)	
Mean ± SD, 1000 cells/μL	—	—	826.7 ± 134.4	355.1 ± 66.4	
WBC					.1
Study arms (patients), n	NA	NA	2 (132)	2 (123)	
Mean ± SD, 1000 cells/μL	—	—	15.1 ± 7.5	0 ± 0	
Kellgren-Lawrence grade 0					.0001
Study arms (patients), n	0	1 (100)	2 (122)	1 (72)	
n/total (%)	0/50 (0)	40/265 (15)	54/182 (30)	31/227 (14)	
Kellgren-Lawrence grade 1, 2, or 3					.0001
Study arms (patients), n	0	3 (205)	3 (182)	4 (227)	
n/total (%)	50/50 (100)	206/265 (78)	104/182 (57)	185/227 (81)	
Kellgren-Lawrence grade 4					.0001
Study arms (patients), n	0	1 (100)	2 (122)	2 (132)	
n/total (%)	0/50 (0)	19/265 (7)	24/182 (13)	11/227 (5)	
Baseline WOMAC					NA
Study arms (patients), n	1 (23)	3 (194)	1 (60)	3 (185)	
Mean	45.5 ± 0	51.4 ± 16.2	38.8 ± 0	55.9 ± 17.1	
Baseline IKDC					NA
Study arms (patients), n	1 (50)	2 (155)	3 (176)	2 (122)	
Mean	55.8 ± 0	50.1 ± 2.1	44.3 ± 3.9	47.5 ± 3.1	

[a]Continuous data are presented as means with SDs; categorical data are presented as frequencies. IKDC, International Knee Documentation Committee subjective score; NA, not available; PRP, platelet-rich plasma; WBC, white blood cell count; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

concentrations than LP-PRP. However, there were statistically significant differences in the distribution of Kellgren-Lawrence grades between groups. The clinical significance of these differences is difficult to infer. On the basis of the study-level data available, we could not distinguish between grades 1, 2, and 3, which represent a wide spectrum of osteoarthritis severity. In addition, all treatment groups had a majority of patients with grades 1, 2, and 3. The HA, LR-PRP, and LP-PRP all had approximately a 2:1 ratio of patients with grade 0 and grade 4, respectively. Therefore, while the LR-PRP group had more patients with radiographically severe disease, it also had more patients with radiographically normal disease than the LP-PRP group. It is therefore difficult to conclude that the severity of disease was a consistent source of bias. Since no firm guidelines have been developed to determine homogeneity thresholds for unbiased NMA,[19] the authors felt that the degree of variation between groups did not preclude NMA. In addition, the difference between all direct and indirect estimates of pairwise comparisons was calculated. The results are shown in Appendix Table A1 (available in the online version of this article at http://ajsm.sagepub.com/supplemental). No significant differences were identified, further confirming the assumption of transitivity and the validity of subsequent NMA.

Results of Network Meta-analysis

Raw data extracted from the eligible studies are shown in Appendix Table A2 (available online). For each outcome variable, a forest plot representing every possible pairwise treatment comparison was created. These results are summarized in Figure 3. Four studies contributed to the WOMAC and IKDC analyses, and 5 studies contributed to the local adverse reaction analysis (Appendix Table A2). Next, treatments were ranked based on each outcome variable using the SUCRA statistic. These results, as well as the probability of each treatment being the best, are

TABLE 4
Treatment Rankings Based on SUCRA Calculations[a]

Variable/Treatment	Rank	SUCRA, %
ΔWOMAC		
Leukocyte-poor PRP	1	98
Placebo	2	41
Leukocyte-rich PRP	3	39.9
Hyaluronic acid	4	21
ΔIKDC		
Leukocyte-poor PRP	1	80.3
Leukocyte-rich PRP	2	75.5
Hyaluronic acid	3	24
Placebo	4	20.2
Adverse events		
Placebo	1	80.2
Hyaluronic acid	2	78.1
Leukocyte-poor PRP	3	22
Leukocyte-rich PRP	4	19.8

[a]For each outcome variable, the surface under the cumulative ranking (SUCRA) was calculated for each treatment option, and treatments were then ranked in order of descending SUCRA. IKDC, International Knee Documentation Committee; PRP, platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

presented in Table 4. Meta-regression was performed on the WOMAC data with baseline WOMAC scores and treatment type as covariates. The *P* value for the baseline WOMAC coefficient was .43, suggesting no statistically significant effect of this variable on the model.

Systemic reactions to PRP were inconsistently reported, which is why quantitative analysis was limited to local adverse reactions. One study reported syncope, dizziness, headache, gastritis, and tachycardia in 17 patients.[36] Although another study reported systemic "adverse events" in one-third of the patients, these included only unrelated conditions that occurred during the study period, including toothache, unrelated trauma or surgery, and urinary tract, abdominal, and upper respiratory infections.[40] Local knee reactions, including transient pain and swelling, were reported consistently in 5 studies.[25,36,40,41,44] An additional study reported postinjection knee pain and swelling, but it was excluded from quantitative analysis since 100% of patients in both treatment groups had these symptoms.[13]

DISCUSSION

This NMA provides a quantitative synthesis of 6 RCTs and 3 prospective comparative trials—including a total of 1055 patients—investigating the use of PRP for OA of the knee. Specifically, we focused on the role of leukocyte concentration on clinical outcome. Overall, the available evidence is of low quality. The principal findings of this study are twofold: (1) functional outcome scores are at best marginally affected by leukocyte concentration, in favor of LP-PRP, and (2) the incidence of local reactions to PRP injections is not affected by leukocyte concentration.

Given the paucity of NMA in the orthopaedic literature, it is important to discuss the subtleties of interpreting these results. First, readers should understand that the most definitive results of NMA are the pairwise odds ratios or mean differences reported in forest plots. These can be interpreted in essentially the same way as traditional pairwise meta-analysis. SUCRA values and treatment rankings require more caution.[39] The SUCRA for a given treatment represents the probability that that treatment is ideal.[39] Therefore, if treatment A has a SUCRA of 80 and treatment B has a SUCRA of 40, treatment A is twice as likely to be the optimal treatment. However, the SUCRA values give no information about the magnitude or clinical significance of the difference between treatments.[10] Using the example above, treatment A might have a success rate of 2% and treatment B a success rate of 1.9%, even with such a large difference in SUCRA.

With these points in mind, it is clear that no significant difference was found in IKDC subjective scores between LR-PRP and LP-PRP. This is consistent with a previous study directly comparing LR-PRP and LP-PRP.[13] While LP-PRP had a slightly higher SUCRA and was therefore ranked first, the confidence interval for the LR-PRP/LP-PRP comparison is wide and crosses the null reference. It is also interesting to note that neither LR-PRP nor LP-PRP showed significantly different effects on IKDC scores compared with HA or placebo injections.

The effect of leukocyte concentration on WOMAC scores was more notable. While the confidence interval on the point estimate comparing LR-PRP and LP-PRP is quite large, this is mainly an effect of the small number of studies available for inclusion in our NMA. What is salient is that LP-PRP demonstrated a significantly greater improvement of WOMAC scores than both placebo and HA, while LR-PRP did not. The magnitude of these differences (17 and 21 points, respectively) is well beyond the minimal clinically important difference for the WOMAC—1.33 points.[1] Unfortunately, there was an imbalance in the baseline WOMAC scores between LR-PRP and LP-PRP groups, with the latter having more severe scores. This is of interest since it has been suggested that PRP is more effective in patients with less symptomatic OA.[13,48] If one assumes that the difference between LR-PRP and LP-PRP groups was actually driven by their baseline WOMAC, the exact opposite would be true in our data. To better understand the contribution of this baseline difference, meta-regression including treatment type and baseline WOMAC scores as independent variables was performed. This did not show a significant influence of the baseline WOMAC scores. However, meta-regression has relatively low power to detect such effects when only a few studies are available for analysis.[19]

Perhaps the most surprising finding of our study is that the leukocyte concentration of PRP does not affect the incidence of local adverse reactions. Preliminary in vitro and animal studies have shown increased proinflammatory characteristics of LR-PRP.[3,11,29,31] It has been

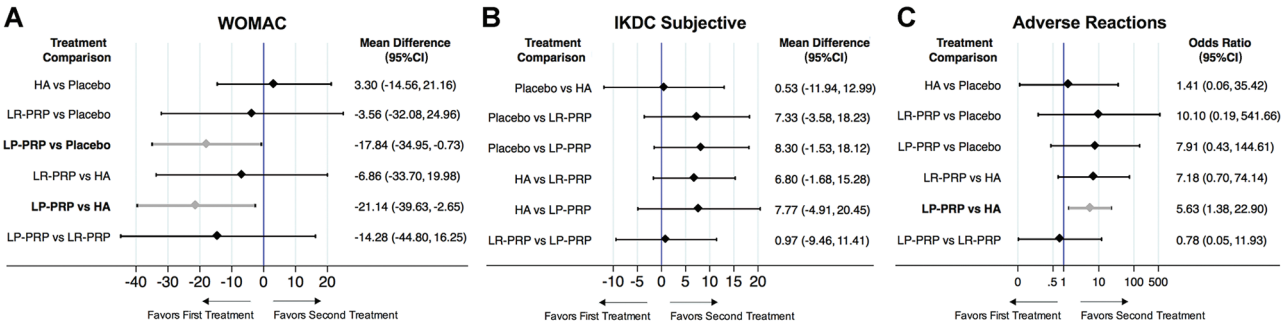

Figure 3. Network meta-analysis results. For each outcome variable, all pairwise treatment comparisons are represented in a forest plot. (A, B) For continuous variables (WOMAC and IKDC scores), treatment effects are reported as mean differences with 95% credible intervals. The vertical reference line represents a mean difference of 0, indicative of statistical equivalence. (C) Similarly, for categorical outcomes (local adverse reactions), treatment effects are reported as odds ratios with 95% credible intervals. The vertical reference line represents an odds ratio of 1, indicative of statistical equivalence. Credible intervals colored in gray do not cross the reference line and therefore represent statistically significant differences. HA, hyaluronic acid; IKDC, International Knee Documentation Committee; LP-PRP, leukocyte-poor platelet-rich plasma; LR-PRP, leukocyte-rich platelet-rich plasma; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

inferred, based on these studies, that the local adverse reactions experienced by patients after PRP knee injections are related to the activation of inflammatory cascades and that LP-PRP preparations should be used to minimize these reactions.[30] In a single prospective comparative study, LR-PRP led to more severe local reactions than LP-PRP.[13] It should be noted, however, that in this study 100% of patients in both treatment groups had knee pain and swelling, and only the subjective severity of these symptoms differed, which is highly susceptible to bias. The actual incidence of local reactions was not different between groups. This is consistent with our results, which suggest that there is no clear relation between leukocyte concentration and the presence of clinically relevant inflammatory reactions. Future studies comparing LR-PRP and LP-PRP are clearly needed and should include standardized definitions of adverse events.

The results of this study are consistent with those of prior meta-analyses.[9,23] Specifically, a small improvement in WOMAC scores and an increase in adverse advents were observed with PRP injections compared with HA and placebo. The effect sizes observed in this study were smaller, likely because of a more stringent statistical design. Indeed, several drawbacks were noted in the previous meta-analyses, including inclusion of low-quality studies[9] and statistical grouping of HA and placebo injections.[9,23] In addition, previous studies treated all PRP preparations as equal, which boosts statistical power by increasing sample sizes but also ignores biological differences between LR-PRP and LP-PRP.

Nonetheless, our study has several limitations. The internal validity of an NMA is determined by the quality and number of studies available to model the statistical model. Only 9 studies were available for analysis, and 3 of these studies provided level 2 evidence. In addition, the outcomes reported were so inconsistent that many interesting variables, including Tegner scores and Knee Injury and Osteoarthritis Outcome Scores, could not be studied. There was statistical heterogeneity in the distribution of Kellgren-Lawrence grades between treatment groups. However, the clinical importance of these differences was questionable. This does raise the question of how strong is the association between the radiographic severity of osteoarthritis and the efficacy of PRP injections. To date, a single randomized study has found a trend toward improved efficacy of PRP in patients with Kellgren-Lawrence grades lower than 2.[12] Further prospective studies are required to answer this question. With respect to external validity, it should be noted that the included studies enrolled young patients (with mean ages in the mid-50s) with predominantly mild OA based on functional scores and radiographic findings. Therefore, the results of this NMA may not be applicable to older patients and those with advanced knee OA. Finally, all but one study[41] included in this analysis used series of PRP injections (typically 3 injections). Therefore, the results may not be applicable to single-injection PRP protocols.

In summary, an NMA of 9 studies (1055 patients), all with level of evidence 1 or 2, investigating the use of PRP for knee OA reveals significant shortcomings in the evidence available to surgeons, physicians, and health care decision makers. Nonetheless, there is evidence that LP-PRP may have a greater effect on functional outcome scores than LR-PRP. Further clinical studies analyzing PRP injections should include information regarding the leukocyte concentration used. In addition, these 2 treatments have similar incidences of local adverse reactions. This study provides justification for a dedicated, larger RCT comparing LR-PRP and LP-PRP in the treatment of knee OA. In addition, since local adverse reactions appear to be a class reaction of PRP that is not dependent on WBC concentration, further laboratory research is warranted to understand the exact mechanism of these reactions and how they can be prevented.

REFERENCES

1. Angst F, Aeschlimann A, Stucki G. Smallest detectable and minimal clinically important differences of rehabilitation intervention with their implications for required sample sizes using WOMAC and SF-36 quality of life measurement instruments in patients with osteoarthritis of the lower extremities. *Arthritis Rheum*. 2001;45(4):384-391.
2. Bibbo C, Bono CM, Lin SS. Union rates using autologous platelet concentrate alone and with bone graft in high-risk foot and ankle surgery patients. *J Surg Orthop Adv*. 2005;14(1):17-22.
3. Boswell SG, Schnabel LV, Mohammed HO, Sundman EA, Minas T, Fortier LA. Increasing platelet concentrations in leukocyte-reduced platelet-rich plasma decrease collagen gene synthesis in tendons. *Am J Sports Med*. 2014;42(1):42-49.
4. Braun HJ, Kim HJ, Chu CR, Dragoo JL. The effect of platelet-rich plasma formulations and blood products on human synoviocytes: implications for intra-articular injury and therapy. *Am J Sports Med*. 2014;42(5):1204-1210.
5. Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *Am J Sports Med*. 2011;39(2):266-271.
6. Cerza F, Carni S, Carcangiu A, et al. Comparison between hyaluronic acid and platelet-rich plasma, intra-articular infiltration in the treatment of gonarthrosis. *Am J Sports Med*. 2012;40(12):2822-2827.
7. Chahal J, Van Thiel GS, Mall N, et al. The role of platelet-rich plasma in arthroscopic rotator cuff repair: a systematic review with quantitative synthesis. *Arthroscopy*. 2012;28(11):1718-1727.
8. Chaimani A, Higgins JP, Mavridis D, Spyridonos P, Salanti G. Graphical tools for network meta-analysis in Stata. *PLoS One*. 2013;8(10):e76654.
9. Chang KV, Hung CY, Aliwarga F, Wang TG, Han DS, Chen WS. Comparative effectiveness of platelet-rich plasma injections for treating knee joint cartilage degenerative pathology: a systematic review and meta-analysis. *Arch Phys Med Rehabil*. 2014;95(3):562-575.
10. Cipriani A, Higgins JP, Geddes JR, Salanti G. Conceptual and technical challenges in network meta-analysis. *Ann Intern Med*. 2013;159(2):130-137.
11. Dragoo JL, Braun HJ, Durham JL, et al. Comparison of the acute inflammatory response of two commercial platelet-rich plasma systems in healthy rabbit tendons. *Am J Sports Med*. 2012;40(6):1274-1281.
12. Filardo G, Kon E, Di Martino A, et al. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. *BMC Musculoskelet Disord*. 2012;13:229.
13. Filardo G, Kon E, Pereira Ruiz MT, et al. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. *Knee Surg Sports Traumatol Arthrosc*. 2012;20(10):2082-2091.
14. Frazer A, Bunning RA, Thavarajah M, Seid JM, Russell RG. Studies on type II collagen and aggrecan production in human articular chondrocytes in vitro and effects of transforming growth factor-beta and interleukin-1beta. *Osteoarthritis Cartilage*. 1994;2(4):235-245.
15. Gobbi A, Lad D, Karnatzikos G. The effects of repeated intra-articular PRP injections on clinical outcomes of early osteoarthritis of the knee [published online April 20, 2014]. *Knee Surg Sports Traumatol Arthrosc*. doi:10.1007/s00167-014-2987-4.
16. Gosens T, Peerbooms JC, van Laar W, den Oudsten BL. Ongoing positive effect of platelet-rich plasma versus corticosteroid injection in lateral epicondylitis: a double-blind randomized controlled trial with 2-year follow-up. *Am J Sports Med*. 2011;39(6):1200-1208.
17. Hart R, Safi A, Komzak M, Jajtner P, Puskeiler M, Hartova P. Platelet-rich plasma in patients with tibiofemoral cartilage degeneration. *Arch Orthop Trauma Surg*. 2013;133(9):1295-1301.
18. Hawkins N, Scott DA, Woods B. How far do you go? Efficient searching for indirect evidence. *Med Decis Making*. 2009;29(3):273-281.
19. Hoaglin DC, Hawkins N, Jansen JP, et al. Conducting indirect-treatment-comparison and network-meta-analysis studies: report of the ISPOR Task Force on Indirect Treatment Comparisons Good Research Practices: part 2. *Value Health*. 2011;14(4):429-437.
20. Hsu WK, Mishra A, Rodeo SR, et al. Platelet-rich plasma in orthopaedic applications: evidence-based recommendations for treatment. *J Am Acad Orthop Surg*. 2013;21(12):739-748.
21. Jansen JP, Naci H. Is network meta-analysis as valid as standard pairwise meta-analysis? It all depends on the distribution of effect modifiers. *BMC Med*. 2013;11:159.
22. Jevsevar DS, Brown GA, Jones DL, et al. The American Academy of Orthopaedic Surgeons evidence-based guideline on: treatment of osteoarthritis of the knee, 2nd edition. *J Bone Joint Surg Am*. 2013;95(20):1885-1886.
23. Khoshbin A, Leroux T, Wasserstein D, et al. The efficacy of platelet-rich plasma in the treatment of symptomatic knee osteoarthritis: a systematic review with quantitative synthesis. *Arthroscopy*. 2013;29(12):2037-2048.
24. Knapp G, Hartung J. Improved tests for a random effects meta-regression with a single covariate. *Stat Med*. 2003;22(17):2693-2710.
25. Kon E, Mandelbaum B, Buda R, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. *Arthroscopy*. 2011;27(11):1490-1501.
26. Leucht S, Cipriani A, Spineli L, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. *Lancet*. 2013;382(9896):951-962.
27. Lippross S, Moeller B, Haas H, et al. Intraarticular injection of platelet-rich plasma reduces inflammation in a pig model of rheumatoid arthritis of the knee joint. *Arthritis Rheum*. 2011;63(11):3344-3353.
28. Magnussen RA, Flanigan DC, Pedroza AD, Heinlein KA, Kaeding CC. Platelet rich plasma use in allograft ACL reconstructions: two-year clinical results of a MOON cohort study. *Knee*. 2013;20(4):277-280.
29. McCarrel T, Fortier L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. *J Orthop Res*. 2009;27(8):1033-1042.
30. McCarrel TM, Mall NA, Lee AS, Cole BJ, Butty DC, Fortier LA. Considerations for the use of platelet-rich plasma in orthopedics. *Sports Med*. 2014;44(8):1025-1036.
31. McCarrel TM, Minas T, Fortier LA. Optimization of leukocyte concentration in platelet-rich plasma for the treatment of tendinopathy. *J Bone Joint Surg Am*. 2012;94(19):e143(141-148).
32. Mont MA, Jacobs JJ, Boggio LN, et al. Preventing venous thromboembolic disease in patients undergoing elective hip and knee arthroplasty. *J Am Acad Orthop Surg*. 2011;19(12):768-776.
33. Nin JR, Gasque GM, Azcarate AV, Beola JD, Gonzalez MH. Has platelet-rich plasma any role in anterior cruciate ligament allograft healing? *Arthroscopy*. 2009;25(11):1206-1213.

34. Noth U, Rackwitz L, Heymer A, et al. Chondrogenic differentiation of human mesenchymal stem cells in collagen type I hydrogels. *J Biomed Mater Res A*. 2007;83(3):626-635.
35. O'Keefe RJ, Crabb ID, Puzas JE, Rosier RN. Effects of transforming growth factor-beta 1 and fibroblast growth factor on DNA synthesis in growth plate chondrocytes are enhanced by insulin-like growth factor-I. *J Orthop Res*. 1994;12(3):299-310.
36. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013;41(2):356-364.
37. Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. *Am J Sports Med*. 2010;38(2):255-262.
38. Radice F, Yanez R, Gutierrez V, Rosales J, Pinedo M, Coda S. Comparison of magnetic resonance imaging findings in anterior cruciate ligament grafts with and without autologous platelet-derived growth factors. *Arthroscopy*. 2010;26(1):50-57.
39. Salanti G, Ades AE, Ioannidis JP. Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. *J Clin Epidemiol*. 2011;64(2):163-171.
40. Sanchez M, Fiz N, Azofra J, et al. A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. *Arthroscopy*. 2012;28(8):1070-1078.
41. Say F, Gurler D, Yener K, Bulbul M, Malkoc M. Platelet-rich plasma injection is more effective than hyaluronic acid in the treatment of knee osteoarthritis. *Acta Chir Orthop Traumatol Cech*. 2013;80(4):278-283.
42. Schepull T, Kvist J, Norrman H, Trinks M, Berlin G, Aspenberg P. Autologous platelets have no effect on the healing of human Achilles tendon ruptures: a randomized single-blind study. *Am J Sports Med*. 2011;39(1):38-47.
43. Schmidt MB, Chen EH, Lynch SE. A review of the effects of insulin-like growth factor and platelet derived growth factor on in vivo cartilage healing and repair. *Osteoarthritis Cartilage*. 2006;14(5):403-412.
44. Spakova T, Rosocha J, Lacko M, Harvanova D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. *Am J Phys Med Rehabil*. 2012;91(5):411-417.
45. Spreafico A, Chellini F, Frediani B, et al. Biochemical investigation of the effects of human platelet releasates on human articular chondrocytes. *J Cell Biochem*. 2009;108(5):1153-1165.
46. Tsai CH, Hsu HC, Chen YJ, Lin MJ, Chen HT. Using the growth factors-enriched platelet glue in spinal fusion and its efficiency. *J Spinal Disord Tech*. 2009;22(4):246-250.
47. Weiner BK, Walker M. Efficacy of autologous growth factors in lumbar intertransverse fusions. *Spine (Phila Pa 1976)*. 2003;28(17):1968-1970; discussion 1971.
48. Zhu Y, Yuan M, Meng HY, et al. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review. *Osteoarthritis Cartilage*. 2013;21(11):1627-1637.