Comparison of humoral insulin-like growth factor-1, platelet-derived growth factor-BB, transforming growth factor-β_1, and interleukin-1 receptor antagonist concentrations among equine autologous blood-derived preparations

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OBJECTIVE
To compare humoral insulin-like growth factor (IGF)-1, platelet-derived growth factor (PDGF)-BB, transforming growth factor (TGF)-β_1, and interleukin-1 receptor antagonist (IL-1Ra) concentrations in plasma and 3 types of equine autologous blood-derived preparations (ABPs).

SAMPLE
Blood and ABP samples from 12 horses.

PROCEDURES
Blood samples from each horse were processed by use of commercial systems to obtain plasma, platelet concentrate, conditioned serum, and aqueous platelet lysate. Half of the platelet concentrate samples were additionally treated with a detergent to release intracellular mediators. Humoral IGF-1, PDGF-BB, TGF-β_1, and IL-1Ra concentrations were measured with ELISAs and compared statistically.

RESULTS
Median IGF-1 concentration was highest in conditioned serum and detergent-treated platelet concentrate, followed by platelet concentrate and plasma; IGF-1 was not detected in platelet lysate. Mean PDGF-BB concentration was highest in platelet lysate, followed by detergent-treated platelet concentrate and conditioned serum; PDGF-BB was not detected in plasma and platelet concentrate. Median TGF-β_1 concentration was highest in detergent-treated platelet concentrate, followed by conditioned serum, platelet lysate, and platelet concentrate; TGF-β_1 was not detected in most plasma samples. Median IL-1Ra concentration was highest in platelet lysate, followed by conditioned serum; IL-1Ra was not detected in almost all plasma, detergent-treated platelet concentrate, and platelet concentrate samples.

CONCLUSIONS AND CLINICAL RELEVANCE
Each ABP had its own cytokine profile, which was determined by the specific processing method. Coagulation and cellular lysis strongly increased humoral concentrations of cell-derived cytokines. No ABP had the highest concentrations for all cytokines. Further studies are needed to assess clinical relevance of these findings. (*Am J Vet Res* 2016;77:898–905)

Various types of ABPs intended as regenerative treatments are frequently used in equine orthopedics. They are prepared by clinicians with each patient's blood and injected locally to promote healing in damaged tissues through the delivery of immunemodulatory and anabolic cell mediators.[1] Numerous commercial systems that involve various processing methods are currently available to generate ABPs. The systems primarily aim to recover and concentrate bioactive platelet-derived molecules (eg, PDGF-BB and TGF-β_1), but certain ABPs also contain IGF-1 (which is mainly found in plasma) and anti-inflammatory cytokines (eg, monocyte-derived IL-1Ra).[2] The additive effects of all these mediators ultimately result in proliferation, stimulation, migration, and differentiation of local and adjacent cells.[3–6] Thus, in horses, ABPs are primarily applied to promote healing of tissues (eg, tendons and ligaments) known for their limited natural capacity for satisfactory regeneration.[7–10] Furthermore, ABPs are gaining in popularity as potential disease-modifying treatments for osteoarthritis.[11–14]

ABBREVIATIONS

ABP	Autologous blood-derived preparation
IGF	Insulin-like growth factor
IL-1Ra	Interleukin-1 receptor antagonist
IQR	Interquartile range
PDGF	Platelet-derived growth factor
TGF	Transforming growth factor

Specific characteristics of end products, such as cell content (cell-rich vs cell-free), type of base solution (plasma, serum, or aqueous solution), and volume, can differ substantially among preparations, depending on the respective processing methods.[2] On the basis of these variables, ABPs can be assigned into 3 main categories: platelet concentrates, conditioned sera, and platelet lysates. Platelet concentrates are cell-rich preparations that sequester and concentrate platelets with or without leukocytes in a relatively small volume of plasma or other base solution.[15] In unstimulated platelet concentrates, most of the cell mediators are located in intracellular granules and only released after platelet activation.[16] In human medicine, exogenous activators are frequently used to increase humoral concentrations of cytokines in platelet concentrates prior to application, whereas in equine medicine, these preparations primarily are applied in an unstimulated form because in situ platelet activation is assumed.[16] Conditioned sera are cell-free, protein-rich, blood-derived solutions obtained after an incubation period. During this process, coagulation occurs and cellular mediators are released from activated blood cells after their contact with glass beads.[17] Because IL-1Ra is enriched, conditioned sera generally are regarded as anticatabolic treatments for osteoarthritis. Additionally, conditioned sera contain anabolic growth factors and can therefore improve tissue healing.[10,17] Platelet lysates are cell-free, protein-rich preparations obtained from platelet concentrates that primarily are subjected to freeze-thaw cycles, osmolar stress, or detergents to induce cellular membrane disruption and passive liberation of the intracellular content of platelets and leukocytes into the base solution before application.[18–20]

In view of the large differences among these preparations with respect to their processing techniques and characteristics, it is possible that they may have extreme differences in individual humoral cell-mediator profiles, which may consequently result in substantial differences in clinical effects. Because the indications for use of these different types of ABPs are overlapping, it is important to first determine their cell-mediator compositions before conducting comparative clinical studies to better understand their specific impact on tissue healing.

The objective of the in vitro study reported here was to compare plasma, 2 forms of a platelet concentrate (unstimulated vs detergent-lysed), conditioned serum, and platelet lysate prepared simultaneously from blood samples obtained from the same horses with regard to humoral IGF-1, PDGF-BB, TGF-β_1, and IL-1Ra concentrations. It was hypothesized that the respective concentrations of these 4 clinically relevant cytokines would differ significantly among the various types of ABPs.

Materials and Methods

Animals

Twelve healthy adult warmblood horses that were part of the teaching herd of the University of Leipzig were included in the study. The horses (10 males and 2 females) were 5 to 16 years old (mean age, 13 years). Complete blood counts were performed to confirm that hematologic values were within reference ranges. Horses were housed individually in box stalls, had free access to hay and water, and did not receive any medication for at least 4 weeks preceding (or during) the study. This study was approved by the legal authorities of Saxony (animal protection authorities of Leipzig) under reference V 04/12, in compliance with the German law of animal welfare.

Preparation of plasma

Skin overlying the left jugular vein of each horse was aseptically prepared. A 14-gauge catheter[a] was inserted into the left jugular vein, and 10 mL of blood was collected into a 12-mL polypropylene syringe loaded with 1 mL of acid-citrate-dextrose A solution.[b] The samples were directly transferred into 15-mL polypropylene tubes and centrifuged in a commercial centrifuge[c] (3,000 × *g* for 10 minutes) to induce cell sedimentation. Cell-free supernatant plasma was collected for measurement of baseline cytokine concentrations.

ABP processing

Three ABPs were simultaneously produced with commercial systems used in accordance with the manufacturers' recommendations. Two forms of a platelet concentrate (unstimulated vs detergent-lysed), conditioned serum, and platelet lysate were obtained.

A commercial system[d] was used to produce a leukocyte-poor platelet concentrate (autologous conditioned plasma). Ten milliliters of blood was obtained from the jugular vein catheter by use of a patented double-syringe loaded with 1 mL of acid-citrate-dextrose A solution. The blood sample was centrifuged (189 × *g* for 5 minutes), and the end product (whole plasma supernatant) was collected in the smaller inner syringe and divided into 2 equal parts. Subsequently, a nonionic detergent solution[e] was added to one of the samples (final detergent concentration, 5%) and allowed to incubate for 20 minutes. This was performed to induce cellular lysis and liberation of intracellular mediators into the detergent-treated plasma concentrate, which therefore enabled us to measure the full intrinsic cytokine concentrations of the plasma concentrate.[18] Both samples (plasma concentrate and detergent-treated plasma concentrate) were then transferred to other tubes[f] and centrifuged by use of a high-speed centrifuge[g] (3,000 × *g* for 10 minutes). The supernatant plasma then was collected with special care to avoid disturbing the pellet at the bottom of the tubes.

Another specific commercial kit[h] was used to prepare conditioned serum. Ten milliliters of blood was collected from the jugular vein catheter into a proprietary syringe that contained borosilicate beads. The syringe was placed in an incubator[i] and incubated at 37°C for 8 hours. The samples were then centrifuged

(3,000 X *g* for 10 minutes), and supernatant was harvested and placed in a 10-mL plastic syringe.

All plasma, plasma concentrate, detergent-treated plasma concentrate, and conditioned serum samples were subsequently filtered by use of syringe filters.[j] Filtered samples were equally divided into 4 cryopreservation tubes,[k] which were subsequently used for determination of humoral cytokine concentrations.

Platelet lysate was also prepared by use of a commercial system.[l] This device consisted of a sterile pyramid-like plastic container with an external lever and 2 ports (an upper inlet and a lower outlet) that were internally connected through a 3-way stopcock coupled to a 0.22-µm filter, both of which were incorporated in the case. Eight milliliters of blood was collected from the jugular vein catheter into a 12-mL polypropylene syringe loaded with 2 mL of 10% hydroxyl ethyl starch and 2 mL of 3.13% sodium citrate. The syringe was placed in a vertical position and allowed to sit undisturbed for 60 minutes to provide for sedimentation. The upper 3.5 mL of the plasma fraction was then gently aspirated into another syringe; this fraction was passed through the inlet of the device to capture the cells in the incorporated filter. Two milliliters of 8.4% sodium hydrogen carbonate was used to flush plasma from the filter into an inner waste reservoir. Subsequently, 1 mL of distilled water was injected through the inlet to induce osmotic lysis of captured cells and rinse the remainder of the sodium hydrogen carbonate from the filter. After 10 minutes of exposure time, a second 2-mL polypropylene syringe was attached to the outlet of the device, and the external lever (attached to the inner 3-way stopcock) was turned to connect the inlet and outlet through the stopcock. Finally, 1 mL of distilled water was injected through the inlet, thereby eluting the cytokine-rich content of the filter into the second syringe. The remaining volume of solution contained in the filter was also aspirated into this second syringe to obtain the final product. The entire process was performed in duplicate to produce a sufficient amount of material. The end product was divided into 4 cryopreservation tubes for subsequent determination of humoral cytokine concentrations.

Measurement of humoral cytokine concentrations

All end products were stored at –80°C until used for measurement of concentrations. One aliquot from each end product was used for determination of 1 cytokine. Human-specific ELISAs were used to measure TGF-β_1[m] and PDGF-BB[n] concentrations. An equine-specific assay[o] was used to determine the IL-1Ra concentration. The IGF-1 concentration was measured with a noncommercial, equine-validated ELISA from the Institute of Veterinary Physiological Chemistry of the University of Leipzig. All measurements were performed in duplicate and in accordance with manufacturer recommendations.

Statistical analysis

Commercial software[p] was used for data analysis. If a cytokine concentration in a sample was below the limit of detection of the respective ELISA kit, the kit-specific threshold value was assigned to this sample to allow statistical analysis. Descriptive statistics and visual inspection of distributions were performed to determine the type of statistical analysis to be applied.[21] When a data set had a normal distribution, mean and SD values were reported for descriptive statistics. When the data did not have a normal distribution, logarithmic transformation was performed to possibly achieve normality. In this case, median and IQR values of the original data set were reported for descriptive statistics. A mixed linear model[q] repeated-measures ANOVA was applied when data had a normal distribution. Tukey tests were used for post hoc pairwise comparisons of the means of each preparation. In the case of truncated distributions (with several values below the detection limit), whereby the prerequisites for an ANOVA were not met, a nonparametric test (Wilcoxon test) was applied. In the case of comparison of data with differently distributed values, comparisons were valid for the order of the nonparametric method, comparison by use of logarithmically transformed values, and analysis by use of original values. Significance was set at values of $P < 0.05$.

Results

Samples

Processing of platelet concentrate was achieved within 10 minutes after collection of blood samples, whereas preparation of platelet lysate and conditioned serum required 1.5 and 8.5 hours after blood sample collection, respectively. Platelet concentrate had the largest volume of end product (6 mL), followed by conditioned serum (4 mL) and platelet lysate (1.4 mL).

IGF-1

Median IGF-1 concentration was highest in conditioned serum (85.75 ng/mL; IQR, 61 to 125.63 ng/mL) and detergent-treated plasma concentrate (85 ng/mL; IQR, 70.13 to 116.63 ng/mL), followed by platelet concentrate (64.5 ng/mL; IQR, 48.25 to 94.75 ng/mL) and plasma (56.75 ng/mL; IQR, 42.75 to 85.63 ng/mL); however, there were no significant differences among these preparations. In contrast, IGF-1 was not detected in platelet lysate samples (< 10 ng/mL); thus, the IGF-1 concentration of platelet lysate differed significantly ($P < 0.001$) from that of the other preparations **(Figure 1)**.

PDGF-BB

Mean ± SD PDGF-BB concentration was highest in platelet lysate (1.35 ± 0.56 ng/mL), followed by detergent-treated plasma concentrate (1.34 ± 0.37 ng/mL) and conditioned serum (1.0 ± 0.25 ng/mL;

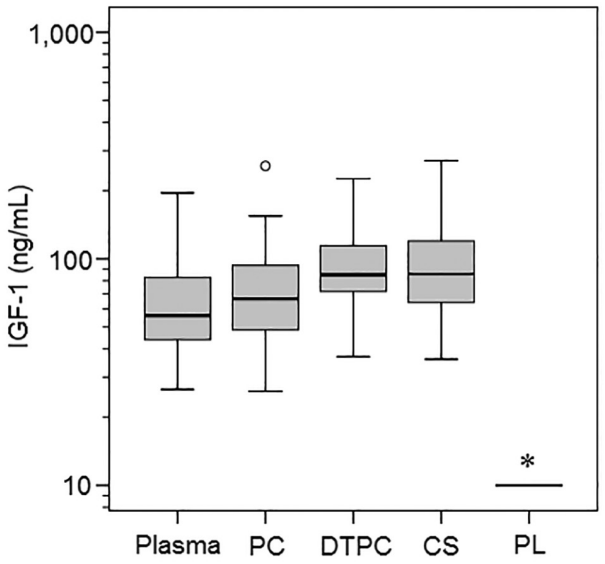

Figure 1—Box-and-whisker plots of the logarithmically transformed humoral IGF-1 concentration in plasma, platelet concentrate (PC), detergent-treated platelet concentrate (DTPC), conditioned serum (CS), and platelet lysate (PL) prepared from blood samples obtained from 12 healthy adult horses. Each box represents the IQR (from the 25th to the 75th percentiles, representing the middle 50% of the data), the horizontal line in each box represents the median, the whiskers indicate the range outside the 25th and 75th percentiles, and circles represent outliers. *Value differs significantly ($P < 0.001$) from the other values.

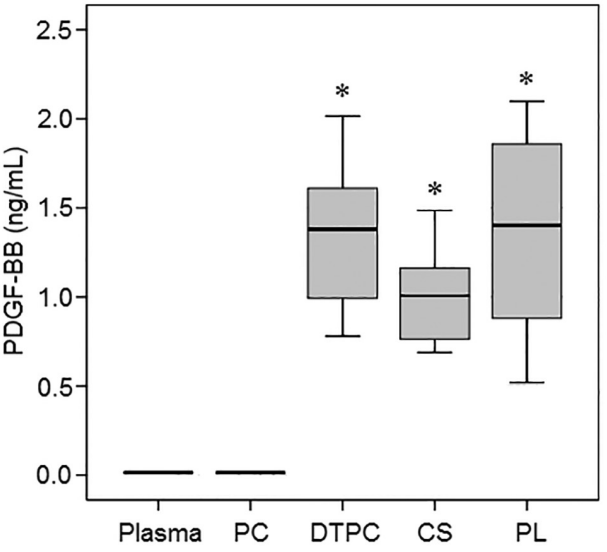

Figure 2—Box-and-whisker plots of the humoral PDGF-BB concentration in plasma, PC, DTPC, CS, and PL prepared from blood samples obtained from 12 healthy adult horses. *Value differs significantly ($P = 0.01$) from the values for plasma and PC. ***See*** Figure 1 for remainder of key.

Figure 2); however, there were no significant differences among these preparations. In contrast, PDGF-BB could not be detected in plasma and platelet concentrate (< 0.015 ng/mL), and the PDGF-BB concentration in these 2 preparations differed significantly ($P < 0.001$) from that of the other preparations.

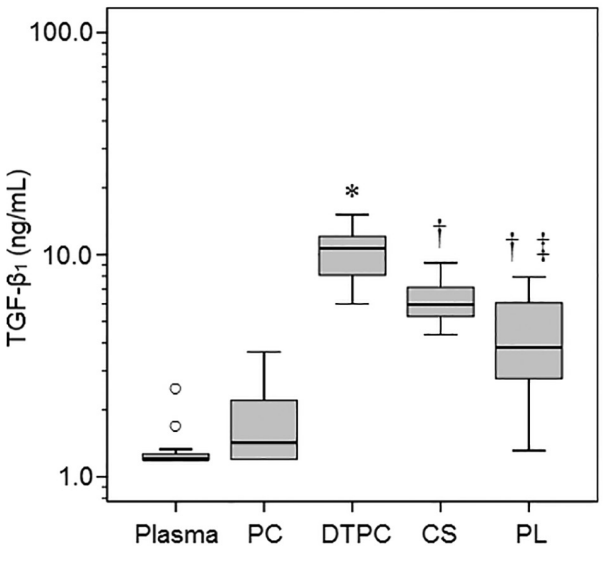

Figure 3—Box-and-whisker plots of the logarithmically transformed humoral TGF-β_1 concentration in plasma, PC, DTPC, CS, and PL prepared from blood samples obtained from 12 healthy adult horses. *Value differs significantly ($P < 0.001$) from the values for all other preparations. †Value differs significantly ($P < 0.001$) from the values for plasma and PC. ‡Value differs significantly ($P < 0.05$) from the value for CS. ***See*** Figure 1 for remainder of key.

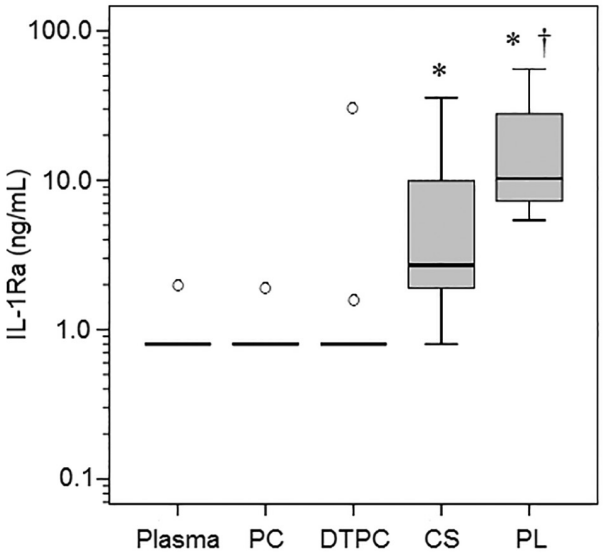

Figure 4—Box-and-whisker plots of the logarithmically transformed humoral IL-1Ra concentration in plasma, PC, DTPC, CS, and PL prepared from blood samples obtained from 12 healthy adult horses. *Value differs significantly ($P = 0.005$) from the values for plasma, PC, and DTPC. †Value differs significantly ($P = 0.01$) from the value for CS. ***See*** Figure 1 for remainder of key.

TGF-β_1

In contrast to the other cytokines, TGF-β_1 was detected in almost all ABPs. Median TGF-β_1 concen-

tration was highest in detergent-treated plasma concentrate (10.68 ng/mL; IQR, 7.67 to 12.28 ng/mL), followed by conditioned serum (5.96 ng/mL; IQR, 5.21 to 7.46 ng/mL), platelet lysate (3.83 ng/mL; IQR, 2.61 to 6.27 ng/mL), and platelet concentrate (1.45 ng/mL; IQR, 1.2 to 2.23 ng/mL). The TGF-β_1 concentration was below the limit of detection in most plasma samples **(Figure 3)**. The TGF-β_1 concentration did not differ significantly ($P < 0.551$) between plasma and platelet concentrate; however, the TGF-β_1 concentration differed significantly among other preparations ($P = 0.024$ between conditioned serum and platelet lysate; $P < 0.001$ between all other preparations).

IL-1Ra

Median IL-1Ra concentration was highest in platelet lysate (10.28 ng/mL; IQR, 7.04 to 31.67 ng/mL), which was followed by conditioned serum (2.71 ng/mL; IQR, 1.86 to 11.5 ng/mL); these values differed significantly ($P = 0.01$). Median IL-1Ra concentration in plasma, platelet concentrate, and detergent-treated plasma concentrate was below the detection limit (< 0.8 ng/mL), except in a few samples **(Figure 4)**. Thus, median IL-1Ra concentrations in platelet lysate and conditioned serum were significantly ($P = 0.005$) higher than in the other preparations.

Discussion

Certain orthopedic disorders, such as osteoarthritis, can be treated with platelet concentrates, conditioned sera, or platelet lysates.[11,12,22] Because substantial differences in cell mediator concentrations between equine products from the same ABP category (eg, between various conditioned sera[17] and between various platelet concentrates[15]) have been reported, large variations in the cytokine profile of preparations in different categories can be expected. To the authors' knowledge, no direct comparison of the respective cell mediator content of various types of ABPs has been reported, although the composition of these preparations influences their effects in tissue healing.[23,24] Results of the study reported here indicated marked discrepancies in the humoral concentrations of IGF-1, PDGF-BB, TGF-β_1, and IL-1Ra between platelet concentrate, conditioned serum, and platelet lysate. To gain a clear understanding of the relevance of these findings, it is important to discuss the basic differences among the 3 preparations before providing an explanation for the variations in concentrations of individual growth factors.

Platelet concentrates primarily segregate and concentrate viable thrombocytes from whole blood samples to deliver their content at the site of injury. In their unstimulated form, humoral platelet-derived mediator concentrations are inferior to total intrinsic values because most of these molecules are located intracellularly.[16,18] Potentially, these preparations have the advantage of a depot-like effect because injected platelets are expected to have a slow and sustained release of their content as well as de novo synthesis of growth factors for up to several days after contact with natural activators.[16,25,26] However, the fate of platelets after local application remains to be elucidated. In 1 study,[13] investigators detected thrombocytes in the synovial fluid of the metacarpophalangeal joints of horses within days after intra-articular injection of platelet concentrate, but the investigators could not distinguish between injected or naturally invading platelets. Moreover, natural in situ activation of the platelet concentrate described in the present study might not be sufficient to induce a satisfactory anabolic tissue effect, as was reported in a placebo-controlled study[27] that involved use of a surgically induced tendon lesion. Although equine platelet concentrates are typically applied without exogenous activation,[2] additional use of activators to induce a stronger effect might be necessary for the platelet concentrate described in the study reported here. In contrast to unstimulated platelet concentrates, conditioned sera and platelet lysates are cell-free products in which the maximal achievable humoral mediator concentrations are reached before application of the preparations (through cellular contact activation and membrane disruption, respectively). Because there is no further release of molecules after injection, a primary depot-like effect may not be expected from these preparations. To ensure a more representative comparison of the total intrinsic cell-mediator content in the 3 types of ABPs, platelet concentrate was additionally lysed with a detergent solution to induce release of intracellular molecules.[18,28]

The base solution has an obvious influence on the cytokine profile of ABPs. Because the platelet concentrate in the present study involved the use of plasma, concentrations of plasma-derived molecules were similar in the samples of platelet concentrate and plasma. In contrast, the base solution of conditioned serum was serum; therefore, this product contained plasma-derived molecules (without coagulation factors) as well as blood cell–derived mediators. Plasma and its specific cytokines were washed out during preparation of platelet lysate. Thus, mediators in platelet lysate, which were dissolved in distilled water, could only originate from the lysed cells caught in the filter.

The ratio between the end volume of ABPs and volume of the initial blood sample is another factor affecting final humoral mediator concentrations. In the platelet concentrate of the present report, final concentrations of cytokines were relatively low because they were first diluted in a relatively large volume (6 mL from a 10-mL initial blood sample, despite an efficient platelet recovery rate [> 90%; data not published]) and then remained mainly within the nonactivated platelets. The quantity of conditioned serum obtained from 10 mL of blood by use of the commercial system was 4 mL. Thus, the dilution effect for cytokines in conditioned serum was less than that of platelet concentrate. In addition, part of the released mediators may have degraded during the long incu-

bation period[29] or remained entrapped in the fibrin clot matrix and not been released in the supernatant serum after centrifugation.[30,31] The platelet lysate kit had the least dilution effect because it yielded 1.4 mL of platelet lysate from 8 mL of blood. This could have explained, at least in part, the relatively high final humoral cytokine concentrations in the platelet lysate. Nevertheless, the ratio between the injected ABP volume and total volume of a treated joint obviously influences final concentrations of the applied cytokines. Thus, the low volume of platelet lysate obtained might not be sufficient to induce satisfactory intra-articular effects in large equine joints in which there will be a large dilution of the cell mediators.[13] Moreover, there may be secondary effusion after ABP injection and further dilution of injected cytokines.[32] In contrast, the end volumes of platelet concentrate and conditioned serum are more adequate for most equine joints, but use of these preparations could lead to painful distention in small joints. Thus, the optimal ABP volumes and mediator concentrations for each joint as well as the fate of injected cytokines or cells (or both) in synovial cavities remains to be determined in further studies.

The cytokine profile of ABPs is influenced by individual patient variability, among other factors.[33–35] In the present study, all blood samples were obtained from the same subjects at the same time, and the end products were prepared simultaneously; thus, differences in specific cell-mediator composition can only be attributed to the respective processing methods. Median IGF-1 concentration in the platelet lysate was below the limit of detection of the ELISA. This finding was expected because the base solution of the specific product was distilled water. Relevance of the lack of IGF-1 on the clinical effect of platelet lysate remains to be determined in in vivo studies. Because IGF-1 is a plasma-specific molecule, concentrations in plasma and unstimulated platelet concentrate were similar, as mentioned previously. Coagulation during preparation of conditioned serum and detergent treatment of the platelet concentrate induced a minor increase in IGF-1 concentrations, compared with the concentrations in plasma and platelet concentrate. This can be explained by the fact that platelets contain small quantities of IGF-1, which is mostly the result of endocytosis from plasma.[36] The PDGF-BB, which is mainly located in α-granules of platelets,[3,37,38] was undetectable in its humoral form in plasma and platelet concentrate, which indicated that there was no relevant platelet activation during centrifugation. Detergent treatment of the platelet concentrate induced passive release of virtually all the platelet contents.[18] The PDGF-BB concentrations were similar in platelet lysate and detergent-treated plasma concentrate and slightly higher than that in conditioned serum. Because detergent-treated plasma concentrate had the largest volume, followed by conditioned serum and platelet lysate, detergent-treated plasma concentrate had the most effective PDGF-BB release. Humoral TGF-β_1 was typically not detected in plasma. Similar to results for PDGF-BB, TGF-β_1 is mainly located in platelets.[3,37,38] Median TGF-β_1 concentration in platelet concentrate was slightly higher than the detection limit. If this slight increase, compared with the TGF-β_1 concentration in plasma, was attributable to some platelet activation during preparation of platelet concentrate, a concomitant release of PDGF-BB would have been expected. Therefore, nonuniform distribution of growth factors in the diverse platelet α-granules and differences in their temporal release kinetics after activation or an inaccuracy in the results of the ELISA measurements can explain this finding.[1,16] Possible explanations for the significantly lower median TGF-β_1 concentration in conditioned serum, compared with the TGF-β_1 concentration in detergent-treated plasma concentrate, were similar to those for PDGF-BB. However, both conditioned serum and detergent-treated plasma concentrate contained significantly higher concentrations of TGF-β_1 than did platelet lysate. Again, this could have been attributable to differences in the release kinetics of both TGF-β_1 and PDGF-BB because TGF-β_1 might have been released earlier than PDGF-BB (before osmotic lysis) and thus washed out during early steps in the preparation of platelet lysate.

With a few exceptions, IL-1Ra was not detected in plasma, platelet concentrate, or detergent-treated platelet concentrate. In blood, IL-1Ra is primarily produced by monocytes,[2] which are greatly decreased in these 3 preparations.[15] Surprisingly, the median humoral concentration of IL-1Ra was significantly higher in platelet lysate than in conditioned serum. After sedimentation was performed during preparation of platelet lysate, a large number of leukocytes, which are rich sources of IL-1Ra,[39] probably were present in the supernatant plasma and consequently trapped in the filter; these leukocytes released their contents, including IL-1Ra, after cell lysis. Unfortunately, hematologic analysis of the supernatant plasma was not performed to confirm this hypothesis. Additionally, as previously mentioned, the dilution effect on cytokine concentrations was much less for platelet lysate. Thus, the absolute amount of IL-1Ra contained in the 4 mL of conditioned serum was higher than that contained in the 1.4 mL of platelet lysate.

The present study had some limitations. First, the ELISA kits used to determine PDGF-BB and TGF-β_1 concentrations were validated for only human plasma and serum by the manufacturer. However, cross-reactivity with equine PDGF-BB and TGF-β_1 has been reported,[40,41] and these kits have been validated for use on equine samples in another study.[42] Second, only 4 cell mediators were measured in the study reported here. Other cell mediators (eg, anabolic vascular endothelial growth factor, fibroblast growth factor, and endothelial growth factor) were not evaluated, even though they are important for the clinical effects of ABPs. Wound healing is an extremely complex process that cannot be adequately described by the ef-

fects of the molecules measured in the present study. Moreover, proinflammatory cytokines (eg, interleukin-1, interleukin-6, or tumor necrosis factor-α) that might negatively influence wound healing were not evaluated in this study. For all these reasons, further laboratory experiments in which concentrations of more cytokines are measured are needed to compare these ABPs. Although the present findings indicated that the clinical effect of these preparations may differ, blinded controlled comparative in vivo and clinical studies are needed to determine whether differences in the composition of these ABPs have an important role in their clinical effects.

The study reported here highlighted the fact that the specific processing method had a strong impact on cell mediator composition and other characteristics of respective end products. Platelet concentrate in the present study had low platelet-derived humoral cytokine concentrations, compared with concentrations in conditioned serum and platelet lysate; thus, exogenous platelet activation might be needed to yield satisfactory clinical results. As expected, detergent treatment (chemical lysis) of platelet concentrate significantly increased humoral mediator concentrations. However, even though this method has been used for clinical treatments in human patients, it cannot yet be recommended for routine clinical use in horses or other domestic animals because the detergent solution might harm local tissues. Although the platelet lysate obtained with the commercial system used in this study contained relatively high concentrations of PDGF-BB, TGF-β_1, and IL-1Ra, it lacked IGF-1 and had a relatively low final volume. This study also confirmed that conditioned serum contained IL-1Ra as well as anabolic proteins. Clinicians should be aware of these differences when selecting an ABP for clinical use in patients. Finally, comparative blinded clinical studies are needed to determine the impact of these in vitro findings on the in vivo effect of platelet concentrate, conditioned serum, and platelet lysate.

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Footnotes

a. Vasofix, B Braun AG, Melsungen, Germany.
b. NoClot-400, Cytosol Laboratories, Braintree, Mass.
c. Rotofix 32A, rotor 1324, Andreas Hettich GmbH, Tuttlingen, Germany.
d. ACP, Arthrex Inc, Naples, Fla.
e. Triton X-100, Sigma-Aldrich Corp, St Louis, Mo.
f. Eppendorf tubes 3810X, Eppendorf AG, Hamburg, Germany.
g. Centrifuge 5415R, Eppendorf AG, Hamburg, Germany.
h. Orthokine vet 10 mL, Orthogen Veterinary GmbH, Düsseldorf, Germany.
i. Melag oHG, Berlin, Germany.
j. Millex GP 0.22-µm, Millipore, Tullagreen, Ireland.
k. Cryotubes 2.0 mL, Techno Plastic Products AG, Trasadingen, Switzerland.
l. ATR, Curasan AG, Kleinostheim, Germany.
m. Human TGF-β1 QKit, R&D Systems Inc, Minneapolis, Minn.
n. Human PDGF-BB Quantikine, R&D Systems Inc, Minneapolis, Minn.
o. Equine IL-1Ra duo set, R&D Systems Inc, Minneapolis, Minn.
p. SAS, version 9.2, SAS Institute Inc, Cary, NC.
q. PROC MIXED, SAS, version 9.2, SAS Institute Inc, Cary, NC.

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