lek. Maciej Dejnek

Kliniczne i molekularne aspekty leczenia entezopatii nadkłykcia bocznego kości ramiennej z użyciem autologicznego osocza bogatopłytkowego

---

ROZPRAWA DOKTORSKA

Promotor: prof. dr hab. n. med. Paweł Reichert

WROCŁAW 2022

Podziękowania

Składam serdeczne podziękowania mojemu promotorowi Panu Profesorowi Pawłowi Reichertowi za umożliwienie rozwoju naukowego, za nieustanną motywację, olbrzymią cierpliwość oraz obdarzenie zaufaniem.

Chciałbym szczególnie podziękować Pani dr hab. Aleksandrze Królikowskiej za nieocenione wsparcie i liczne cenne uwagi merytoryczne, które wprowadziły realizowane przeze mnie projekty badawcze na zdecydowanie wyższy poziom.

Pracę dedykuję mojej kochanej Żonie Bogusi dziękując za miłość, cierpliwość i wsparcie, którymi obdarza mnie na co dzień i bez których powyższa praca by nigdy nie powstała.

SPIS TREŚCI

I. LISTA PUBLIKACJI WCHODZĄCYCH W SKŁAD CYKLU PRAC 4

II. OMÓWIENIE PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY 5

WSTĘP 5

CELE 7

METODYKA 8

WYNIKI 10

WNIOSKI 12

BIBLIOGRAFIA 14

III. PRACA NR 1: *Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study* 17

IV. PRACA NR 2: *Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods* 26

V. PRACA NR 3: *Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses* 46

VI. PRACA NR 4: *Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma* 59

VII. STRESZCZENIE 82

VIII. ABSTRACT 84

IX. ZAŁĄCZNIKI 86

1. OŚWIADCZENIA WSPÓŁAUTORÓW 86
2. ŹRÓDŁA FINANSOWANIA 101
3. ZGODY KOMISJI BIOETYCZNEJ 102

I. LISTA PUBLIKACJI WCHODZĄCYCH W SKŁAD CYKLU PRAC

1. **Maciej Dejnek**, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert.

   Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study

   Advances in Clinical and Experimental Medicine 2021; 30(7):757–764.
   https://doi.org/10.17219/acem/135045
   IF_{2021} = 1,736; pkt MNiSW: 70,00

2. **Maciej Dejnek**, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska.

   Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods.

   World Journal of Orthopedics 2022; 13(6): 587-602.
   https://doi.org/10.5312/wjo.v13.i6.587
   IF_{2021} = 0; pkt MNiSW: 140,00

3. **Maciej Dejnek**, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska.

   Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses

   Oxidative Medicine and Cellular Longevity 2022; 2022, 8059622.
   https://doi.org/10.1155/2022/8059622
   IF_{2021} = 7,310; pkt MNiSW: 100,00

4. **Maciej Dejnek**, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska.

   Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma

   Journal of Clinical Medicine 2022, 11(13), 3687.
   https://doi.org/10.3390/jcm11133687
   IF_{2021} = 4,964; pkt MNiSW: 140,00

Sumaryczny IF_{2021} = 14,01
Suma pkt MNiSW = 450,00

II. OMÓWIENIE PUBLIKACJI WCHODZĄCYCH W SKŁAD ROZPRAWY

WSTĘP

Entezopatia nadkłykcia bocznego kości ramiennej jest częstym schorzeniem objawiającym się poprzez silne dolegliwości po bocznej stronie stawu łokciowego. Dotyka ono nawet 1-3% populacji ogólnej, z podobną częstotliwością mężczyzn i kobiet, głównie pomiędzy 40. a 60. rokiem życia [1]. Choroba ta, powszechnie znana jako „łokieć tenisisty", dotyka nie tylko sportowców ale przede wszystkim pracowników narażonych na przeciążenia kończyny górnej. Najlepiej udokumentowanymi czynnikami ryzyka są wielokrotnie powtarzające się w ciągu dnia ruchy zgięcia i wyprostu nadgarstka, ruchy skrętne przedramienia oraz dźwiganie ciężkich przedmiotów [2]. Osoby dotknięte tą przypadłością mają problemy nie tylko z wykonywaniem aktywności sportowej ale również z wykonywaniem pracy, a nawet prostych czynności dnia codziennego. Z histopatologicznego punktu widzenia podłożem dolegliwości bólowych jest angiofibroblastyczna degeneracja bliższego przyczepu ścięgien prostowników przedramienia do nadkłykcia bocznego kości ramiennej. Wielokrotnie powtarzające się mikrouszkodzenia w obrębie zajętej tkanki zaburzają procesy naprawcze, a początkowy stan zapalny stopniowo przeistacza się w proces degeneracyjny z zaburzeniem architektury włókien przyczepu ścięgna [3]. Choroba ma charakter samoograniczający się i u większości pacjentów ustępuje samoistnie w przeciągu roku [2]. Podczas tego okresu dolegliwości bólowe mogą być jednak na tyle dokuczliwe, iż pacjenci aktywnie poszukują rozwiązań mogących przynieść ulgę w cierpieniu. Leczeniem pierwszego rzutu jest oszczędzanie kończyny, zmiana wzorca ruchowego, ćwiczenia rozciągające i wzmacniające oraz fizykoterapia [4]. W przypadku, gdy tego typu postępowanie nie przyniesie poprawy, powszechną praktyką jest ordynowanie iniekcji glikokortykosteroidowych w okolicę przyczepu prostowników do nadkłykcia bocznego kości ramiennej. Pomimo początkowej wysokiej skuteczności leczenie to wiąże się ze zwiększonym odsetkiem nawrotów oraz ryzykiem licznych powikłań [5].

Jako alternatywną metodę terapeutyczną ukierunkowaną na stymulację naturalnych procesów regeneracyjnych zaproponowano iniekcje z autologicznego osocza bogatopłytkowego (PRP) [6]. PRP można zdefiniować jako osocze o zawartości płytek krwi powyżej jednego miliona na mikrolitr (µL) [7]. Nie wszystkie dostępne protokoły wytwarzania PRP pozwalają jednak na osiągnięcie takiego zagęszczenia. Amerykańska Agencja Żywności i Leków (FDA) pozwala na używanie terminu „PRP" do produktów o zawartości płytek krwi przekraczającej 250 tysięcy na µL. Część badaczy dowodzi, że ilość płytek krwi powyżej 200 tysięcy na µL jest wystarczająca aby uzyskać efekt terapeutyczny [8]. Taka definicja wprowadza jednak pewne zamieszanie, ponieważ jest to wartość płytek krwi znajdująca się w granicach normy dla krwi pełnej w zdrowej populacji. Idea leczenia przy pomocy PRP polega na wyizolowaniu z krwi pacjenta frakcji osocza bogatej w płytki krwi, które po podaniu w chorobowo zmienione miejsce uwalniają ze swoich ziarnistości typu alfa, znaczne ilości czynników wzrostu [6]. Do najważniejszych i najlepiej poznanych należą transformujący czynnik wzrostu beta-1 (TGF-β1), naskórkowy czynnik wzrostu (EGF),

czynnik wzrostu fibroblastów (FGF), naczyniowo-śródbłonkowy czynnik wzrostu (VEGF), czynnik wzrostu hepatocytów (HGF) oraz płytkopochodne czynniki wzrostu (PDGF). Cytokiny tego typu są odpowiedzialne za przebieg prawidłowych procesów naprawczych w organizmie poprzez regulację metabolizmu komórkowego, odpowiedzi zapalnej, stymulację angiogenezy, chemotaksję makrofagów, monocytów czy fibroblastów do miejsca uszkodzenia, stymulację proliferacji oraz różnicowania keratynocytów i fibroblastów [9]. Zawartość leukocytów w PRP również nie pozostaje bez znaczenia. Z jednej strony leukocyty pozytywnie korelują z zawartością pożądanych czynników wzrostu, modulują odpowiedź immunologiczną oraz posiadają właściwości zwalczania drobnoustrojów. Z drugiej jednak strony mogą wywierać negatywny wpływ na procesy gojenia stymulując procesy kataboliczne poprzez lokalne uwalnianie cytokin prozapalnych oraz licznych proteinaz [10, 11].

Wiele badań potwierdzających skuteczność i bezpieczeństwo PRP w leczeniu trudno gojących się ran, opóźnionego zrostu kostnego oraz urazów sportowych zachęciło do jego powszechnego stosowania [12, 13]. Niestety wraz ze wzrostem ilości wysokiej jakości badań na większych grupach pacjentów pojawiły się liczne kontrowersje co do skuteczności metody [14]. PRP przygotowane przy pomocy różnych technik potrafią różnic się znacznie pod względem zawartości i koncentracji składników komórkowych a tym samym pożądanych cytokin stymulujących procesy naprawcze. Powyższe różnice wynikać mogą z takich zmiennych jak ilość pobranej krwi, budowa separatora, prędkość i ilość wirowań, metoda ekstrakcji osocza, sposoby aktywacji płytek krwi, zastosowanie dodatkowych substancji czynnych (np. środków antykoagulujących lub buforujących) [15]. Kolejnym problemem są różnice w wyjściowej zawartości płytek we krwi pacjentów wynikające z szerokiego zakresu wartości uznanych jako norma populacyjna. Nie bez znaczenia ma również sama technika aplikacji i różnice w zastosowaniu środków znieczulających, objętość podanego PRP, rozmiar igły, liczba nakłuć oraz precyzja podania. Wszystkie powyższe czynniki mogą być przyczyną znacznych rozbieżności w uzyskiwanych wynikach badań klinicznych.

Próbując rozwiązać problem heterogeniczności leczenia z użyciem PRP stworzono wiele klasyfikacji. Pierwsza z nich dzieliła autologiczne osocze bogatopłytkowe na cztery grupy w zależności od zawartości leukocytów oraz fibryny [16]. Z uwagi na to, że nie dostarczała ona wystarczającej ilości niezbędnych informacji powstawały kolejne, coraz bardziej skomplikowane klasyfikacje. Jedną z nich jest klasyfikacja MARSPILL, zaproponowana w 2017 roku, która dzieli preparaty PRP w zależności od sposobu przygotowania (ręczny lub mechaniczny), sposobu aktywacji, obecności czerwonych krwinek, ilości wirowań, uzyskanego zagęszczenia płytek krwi względem wartości wyjściowych, wykorzystania ultrasonografii podczas iniekcji, obecności białych krwinek i uzyskanego ich zagęszczenia [17]. Kryteria powyższej klasyfikacji doskonale obrazują złożoność aspektów klinicznych i laboratoryjnych branych pod uwagę w zakresie leczenia biologicznego w ortopedii z użyciem PRP.

Występowanie istotnych różnic w składzie PRP przygotowanego różnymi sposobami zostało już wcześniej udowodnione przez innych badaczy w licznych publikacjach. Większość tych badań opiera się jednak na próbie o niewielkiej liczebności, porównując zwykle 2-4 metody z kilkunastu dostępnych na rynku oraz analizując niewielką liczbę

parametrów molekularnych [15, 18]. W związku z powyższym istnieje uzasadniona potrzeba dalszego poszerzania wiedzy na temat charakteru różnic PRP uzyskanego z pomocą różnych technik. Było to jedną z przyczyn przeprowadzenia pierwszego badania porównującego cztery z powszechnie stosowanych w Polsce zestawów komercyjnych do przygotowywania PRP w warunkach ambulatoryjnych, z czego dwie metody nie były wcześniej badane pod tym kątem [19]. Znacznie poszerzono również ilość badanych parametrów w stosunku do dotychczas opublikowanych badań, m.in. zwiększając ilość analizowanych czynników wzrostu i cytokin zapalnych oraz analizując powtarzalność otrzymywanych stężeń [20]. Niewielka ilość publikacji porównujących skład molekularny osocza bogatopłytkowego do osocza wyjściowego była przyczyną kontynuacji badań celem poszerzenia analizowanego zakresu parametrów [21].

Najbardziej interesującym aspektem prowadzonych prac była próba ustalenia czy zawartość poszczególnych składników biologicznie aktywnych w PRP w sposób istotny koreluje ze skutecznością leczenia entezopatii nadkłykcia bocznego kości ramiennej [22]. Większość dotychczasowych badań opisujących efekt tkankowy różnych cytokin płytkopochodnych to badania przedkliniczne. Zadecydowały one o zasadności prób leczenia pacjentów z wykorzystaniem iniekcji PRP. W żywym organizmie ludzkim zachodzi jednak znacznie więcej procesów metabolicznych mogących wpływać na ostateczny efekt miejscowego podawania złożonego koktajlu czynników wzrostu jakim jest PRP. Do tej pory opublikowano tylko kilka prac oceniających zależność pomiędzy stężeniem poszczególnych czynników wzrostu w PRP a skutecznością kliniczną leczenia [23-25]. Spośród powyższych publikacji tylko jedna oceniała efektywność leczenia entezopatii nadkłykcia bocznego kości ramiennej [23]. Do tej pory żadna praca nie oceniała zależności pomiędzy zawartością cytokin zapalnych w PRP a jego skutecznością kliniczną.

Iniekcje PRP są obecnie powszechnie stosowane w leczeniu „łokcia tenisisty” na całym świecie, pomimo wciąż niejednoznacznych dowodów, które uzasadniałoby takie postępowanie. Zaprojektowane i przeprowadzone badania wchodzące w skład niniejszej rozprawy doktorskiej miały z założenia w sposób istotny przyczynić się do poszerzenia wiedzy na temat tej metody leczniczej wspomagając proces decyzji terapeutycznych oraz wskazując kierunek dalszych badań dążących do jej udoskonalenia.

CELE

Do głównych celów badawczych pracy doktorskiej należały:

- ustalenie różnic w zawartości składników biologicznie aktywnych takich jak komórki krwi, czynniki wzrostu i cytokiny zapalne w PRP przygotowanym z pomocą różnych technik,

- ocena ilościowych i jakościowych różnic pomiędzy uzyskanym PRP a surowicą pacjentów pod względem zawartości składników biologicznie aktywnych,

- ustalenie zależności pomiędzy zawartością składników biologicznie aktywnych w PRP a skutecznością leczenia iniekcyjnego pacjentów z entezopatią nadkłykcia bocznego kości ramiennej.

Dodatkowo postawione cele pomocnicze:

- wytypowanie do badań klinicznych jednej z powszechnie dostępnych komercyjnie metod pozwalającej w sposób powtarzalny na przygotowanie PRP o najwyższej zawartości płytek krwi oraz czynników wzrostu,

- ocena zależności pomiędzy zawartością komórkową w PRP a stężeniem wybranych czynników wzrostu oraz cytokin zapalnych,

- ocena zależności pomiędzy czynnikami populacyjnymi jak płeć, wiek oraz wskaźnik masy ciała (BMI) na zawartość wybranych składników biologicznie aktywnych w PRP,

- ocena skuteczności i bezpieczeństwa leczenia iniekcyjnego z użyciem PRP pacjentów z entezopatią nadkłykcia bocznego kości ramiennej.

METODYKA

Aby osiągnąć założone cele w pierwszym badaniu porównano zawartość składników biologicznie aktywnych w PRP przygotowanym z użyciem czterech różnych protokołów [19]. Do tego celu wybrano cztery różne, powszechnie dostępne komercyjne zestawy do przygotowywania autologicznego osocza bogatopłytkowego: 1) Arthrex Autologous Conditioned Plasma (ACP) Double Syringe System (Arthrex Inc., USA), 2) Mini GPS III Platelet Concentration System (Biomet Inc., USA), 3) Xerthra PRP kit (Biovico Sp. z o.o., Polska), 4) Dr. PRP (Rmedica, Republika Korei). Analizę składników komórkowych w krwi pełnej oraz w przygotowanych próbkach PRP wykonano przy pomocy automatycznego analizatora laboratoryjnego Mindray BC-5150 (Shenzhen Mindray Bio-Medical Electronics Co., Chińska Republika Ludowa). Oceniono zawartość płytek krwi, erytrocytów oraz białych krwinek i ich podtypów. Stężenie wybranych czynników wzrostu w PRP oceniono przy pomocy immunofluorescencyjnej metody multipleksowej LEGENDplex™ (BioLegend, USA) i cytometrii przepływowej. Aby otrzymać homogenną grupę do badania pilotażowego zaproszono sześciu zdrowych ochotników płci męskiej w wieku 27-28 lat. Czynniki wzrostu, których stężenie oceniono w tym badaniu to TGF-β1, EGF, VEGF oraz płytkopochodny czynnik wzrostu AA (PDGF-AA). Przy pomocy analizy statystycznej porównano różnice zawartości komórkowej w krwi pełnej oraz próbkach PRP. Porównano skład komórkowy, zawartość czynników wzrostu oraz efektywność wychwytu płytek krwi w PRP uzyskanym za pomocą analizowanych protokołów. Oszacowano również występowanie korelacji pomiędzy składnikami komórkowymi w PRP a stężeniem wybranych czynników wzrostu.

W kolejnym etapie badanie poszerzono grupę do 12 ochotników płci męskiej w wieku 24-35 lat, zwiększając również ilość badanych czynników wzrostu oraz oceniając panel trzynastu cytokin zapalnych: interferon alfa-2 i gamma (IFN-α2, IFN-γ), czynnik martwicy nowotworu alfa (TNF-α), białko chemotaktyczne monocytów (MCP-1) oraz interleukiny (IL-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33) [20]. Spośród czynników wzrostu, względem badania pilotażowego, dodatkowo oceniono zawartość podstawowego czynnika wzrostu fibroblastów (FGF-basic), HGF oraz płytkopochodnego czynnika wzrostu BB (PDGF-BB). Zbadano również powtarzalność uzyskiwanego składu komórkowego PRP w różnych metodach jego pozyskiwania.

W kolejnej pracy przeprowadzono analizę próbek krwi pełnej, surowicy oraz PRP uzyskanych od 31 pacjentów w wieku 35-60 lat (15 mężczyzn, 16 kobiet) leczonych z powodu entezopatii nadkłykcia bocznego kości ramiennej [21]. Porównano w zebranym materiale biologicznym zawartość składników komórkowych oraz stężenie badanych wcześniej siedmiu czynników wzrostu i trzynastu cytokin zapalnych. Przy pomocy analizy statystycznej oceniono istotność występujących różnic w zawartości składników biologicznie aktywnych pomiędzy PRP a wartościami bazowymi w krwi pełnej i surowicy oraz zależność pomiędzy nimi a wiekiem i BMI pacjentów. Zbadano również występowanie korelacji pomiędzy składnikami komórkowymi a badanymi cytokinami w PRP.

W badaniu klinicznym przeprowadzono leczenie iniekcyjne 30 pacjentów w wieku 35-60 lat z entezopatią nadkłykcia bocznego kości ramiennej [22]. W badaniu oceniono zależności pomiędzy zawartością komórkową, stężeniem czynników wzrostu oraz cytokin zapalnych w PRP a skutecznością kliniczną leczenia badaną w trakcie wizyt kontrolnych po 1 i 3 miesiącach od iniekcji. Skuteczność leczenia oceniano poprzez oszacowanie zmiany natężenia dolegliwości bólowych mierzonych w wizualnej skali analogowej (VAS) jako średnia na dany dzień oraz podczas wykonywania testów prowokacyjnych (test Cozena, test Milla, test Maudsley'a, test Thomsona oraz test krzesła). Celem obiektywizacji natężenia dolegliwości bólowych wykonano dodatkowo badanie algometrem – Wagner FPIX 25 Pain Test Algometer (Wagner Instruments, USA) poprzez zmierzenie siły jaką trzeba przyłożyć w miejscu zmienionym chorobowo aby wywołać najmniejsze wrażenie bólowe. Oceniono również zmianę siły wybranych grup mięśniowych kończyny górnej z użyciem ręcznego dynamometru microFET2 (Hoggan Scientific, USA) oraz zmianę siły uścisku aparatem BIMS Digital Grip Dynamometer (Baseline, USA). Badani wypełnili polskie wersje kwestionariuszy funkcjonalnych dedykowanych schorzeniom kończyny górnej: Disability of Arm, Shoulder and Hand (DASH) oraz Patient-Rated Tennis Elbow Evaluation (PRTEE). Dodatkowo poproszono pacjentów o subiektywną ocenę stawu łokciowego (SEV) w skali od 0 do 100%, przy założeniu że zupełnie zdrowy staw łokciowy powinien otrzymać wynik maksymalny. Leczenie uznano za skuteczne jeżeli pacjent osiągnął minimalną klinicznie istotną różnicę (MCID) pomiędzy wartością bazową a otrzymaną podczas wizyt kontrolnych wynoszącą 1,5 punktu dla skali VAS, 15,8 punktów dla skali DASH oraz 11 punktów dla skali PRTEE.

Powyższe omówienie zawiera najważniejsze elementy metodologiczne przeprowadzonych badań wchodzących w skład niniejszej rozprawy doktorskiej. Szczegóły

metodologii poszczególnych badań opisane są we właściwych częściach publikacji załączonych w kolejnych rozdziałach.

WYNIKI

W badaniu pilotażowym, porównującym PRP uzyskane za pomocą czterech komercyjnych zestawów stwierdzono występowanie między nimi istotnych różnic. Stężenie płytek krwi było istotnie wyższe w PRP uzyskanym z użyciem Mini GPS III niż Arthrex ACP lub Xerthra PRP kit ($p < 0,05$). Podobnie stężenie leukocytów było istotnie wyższe w PRP przygotowanym przez Mini GPS III niż Arthrex ACP lub Dr.PRP ($p < 0,05$). Najwyższa zawartość czerwonych krwinek występowała w PRP Mini GPS III co istotnie różniło go od pozostałych badanych systemów ($p < 0,05$). Najwyższy wskaźnik efektywności wychwytu płytek również osiągnął Mini GPS III co istotnie różniło go od Xerthra PRP kit ($p = 0,001$) oraz Dr.PRP ($p < 0,05$), ale nie w sposób istotny od Arthrex ACP ($p = 0,061$). Porównując zawartość czynników wzrostu stwierdzono występowanie istotnych różnic pomiędzy dwoma systemami – Mini GPS III oraz Arthrex ACP, tylko w zakresie stężenia EGF ($p < 0,05$) oraz PDGF-AA ($p < 0,05$), które były wyższe w PRP przygotowanym z użyciem pierwszego wymienionego. Pod względem powyższych parametrów pozostałe systemy nie różniły się między sobą w sposób istotny. Badając korelacje pomiędzy zawartością składników komórkowych w PRP a stężeniem badanych czynników wzrostu stwierdzono występowanie istotnej statystycznie, dodatniej korelacji Spearmana pomiędzy stężeniem płytek krwi a EGF ($r = 0,46$) oraz PDGF-AA ($r = 0,58$), pomiędzy stężeniem białych krwinek a PDGF-AA ($r = 0,51$), a także pomiędzy stężeniem czerwonych krwinek a EGF ($r = 0,51$), VEGF ($r = 0,53$) oraz PDGF-AA ($r = 0,57$).

Po podwojeniu populacji z badania pilotażowego oraz zwiększeniu ilości badanych cytokin parakrynnych ponownie stwierdzono najwyższe stężenie składników komórkowych w PRP uzyskanym za pomocą Mini GPS III co w sposób istotny różniło go od pozostałych trzech badanych protokołów. System ten pozwalał na uzyskanie największego zagęszczenia płytek krwi względem wartości bazowych (5,05 x), najwyższego stężenia pięciu z siedmiu badanych czynników wzrostu (EGF, VEGF, HGF, PDGF-AA, PDGF-BB), oraz najwyższego poziomu PCE (56,15% ±7,44%). Największą powtarzalność uzyskiwanych stężeń płytek krwi mierzoną współczynnikiem zmienności (CV) uzyskano systemami Arthrex ACP (CV = 12,18%) oraz Mini GPS III (CV = 13,25%) co istotnie odróżniało te systemy od dwóch pozostałych – Dr.PRP (CV = 34,05) i Xerthra PRP kit (CV = 95,95%).

Spośród oznaczonych czynników wzrostu oraz cytokin zapalnych istotne statystycznie różnice ($p < 0,05$) osiągnięto porównując PRP przygotowane przez Mini GPS III i Arthrex ACP w zakresie stężeń EGF, VEGF, HGF, PDGF-AA, IL-8, IL-18 a także pomiędzy Mini GPS III i Xerthra PRP kit w zakresie stężeń EGF, PDGF-AA, IL-8, IL-18. Stężenie IL-18 było również istotnie wyższe w PRP przygotowanym z użyciem Mini GPS III niż Dr.PRP. Pozostałe protokoły nie różniły się istotnie w zakresie uzyskanych stężeń czynników wzrostu oraz cytokin zapalnych. Potwierdzono wyznaczone w badaniu pilotażowym istotne statystycznie dodatnie korelacje Spearmana pomiędzy zawartością płytek krwi, białych krwinek i erytrocytów a czynników wzrostu takich jak EGF, VEGF, PDGF-AA. Dodatkowo

wykazano obecność istotnej dodatniej korelacji pomiędzy stężeniem składników komórkowych a stężeniem HGF, PDGF-BB, IL-1β, MCP-1, IL-8, IL-18.

Kolejne badanie oceniające różnice pomiędzy wartościami bazowymi w krwi pełnej i osoczu a próbkami PRP przygotowanymi z użyciem systemu Mini GPS III u 31 pacjentów wykazało występowanie istotnego wzrostu zawartości płytek krwi (4,41 x), białych krwinek (4,59 x), TGF-β1 (3,79 x), EGF (2,5 x), PDGF-AA (6,19 x), PDGF-BB (6,43 x), IFN-α2 (2,4 x), IFN-γ (1,39 x), TNF-α (2,12 x), IL-1β (2,99 x), IL-6 (1,52 x), IL-8 (5,89 x), IL-10 (1,46 x), IL-12p70 (1,62 x). W sposób istotny zmniejszyły się w PRP stężenia czerwonych krwinek (0,19 x), FGF-basic (0,68 x), HGF (0,51 x). Nie wykazano istotnych różnic w zawartości VEGF oraz pozostałych cytokin zapalnych pomiędzy PRP a surowicą krwi. W grupie mężczyzn (w porównaniu do grupy kobiet) stwierdzono istotnie wyższą liczbę czerwonych krwinek w krwi pełnej (5,15 vs. 4,56; $p \leq 0,001$), białych krwinek w PRP (34,64 vs. 25,83; $p < 0,05$), a także wyższe stężenia większości z badanych cytokin zapalnych w PRP. Wiek i BMI nie miały istotnego wpływu na zawartość płytek krwi, białych i czerwonych krwinek oraz czynników wzrostu w PRP. Spośród cytokin zapalnych w PRP stwierdzono istotne ujemne korelacje pomiędzy wiekiem a IL-1β ($r = - 0,50$; $p < 0,05$), IFN-α2 ($r = - 0,38$; $p < 0,05$), oraz TNF-α ($r = - 0,39$; $p < 0,05$). BMI z kolei dodatnio korelowało z zawartością IL-1β ($r = 0,40$; $p < 0,05$).

Zgodnie z przewidywaniami potwierdzono dodatnie korelacje pomiędzy zawartością płytek krwi i leukocytów w krwi pełnej a przygotowanymi z nich próbkami PRP. Zawartość płytek krwi w PRP istotnie dodatnio korelowała ze stężeniem EGF ($r = 0,74$; $p \leq 0,001$), PDGF-AA ($r = 0,77$; $p \leq 0,001$), PDGF-BB ($r = 0,79$; $p \leq 0,001$) oraz VEGF ($r = 0,46$; $p < 0,05$). Zawartość leukocytów w PRP istotnie dodatnio korelowała ze stężeniem VEGF ($r = 0,69$; $p \leq 0,001$), HGF ($r = 0,42$; $p < 0,05$), IL-8 ($r = 0,45$; $p <0,05$). Nie stwierdzono występowania istotnej korelacji pomiędzy zawartością płytek krwi a stężeniem cytokin zapalnych w PRP.

W badaniu klinicznym po trzech miesiącach od iniekcji leczenie okazało się skuteczne (osiągnięto MCID) w zakresie redukcji dolegliwości bólowych u 67%, a w zakresie poprawy funkcjonalnej u 83% pacjentów. Jedynym stwierdzonym skutkiem ubocznym zastosowanej terapii była intensyfikacja dolegliwości bólowych u pięciu pacjentów po jednym tygodniu, u czterech pacjentów po jednym miesiącu oraz u dwóch pacjentów po trzech miesiącach, z czego jeden wymagał leczenia operacyjnego. Wiek pacjentów, BMI oraz czas trwania objawów nie korelowały w sposób istotny z uzyskaną poprawą. Średnie zmniejszenie natężenia dolegliwości bólowych w dniu ostatniej kontroli względem wartości wyjściowych wynosiło 2,7 ±2,73 pkt w skali VAS ($p \leq 0,001$), a podczas różnych testów prowokacyjnych średnio od 1,72 do 3,77 pkt ($p < 0,05$). Po trzech miesiącach siła jaką należało przyłożyć algometrem w okolicę przyczepu ścięgien prostowników do nadkłykcia bocznego kości ramiennej aby wywołać dolegliwości bólowe zwiększyła się średnio z 25,38 ±11,76 niutona (N) do 30,44 ±14,28 N ($p < 0,05$). W sposób istotny poprawiła się również siła mięśniowa podczas prostowania nadgarstka (z 116,44 ±52,47 N do 140,46 ±49,45 N, $p < 0,05$), pronacji przedramienia (z 36,64 ±18,35 N do 46,44 ±20,15 N; $p \leq 0,001$) oraz siła uścisku (z 32,45 ±14,28 MAX kg do 35,96 ±14,46 MAX kg; $p < 0,05$). W końcowym okresie obserwacji

stwierdzono również poprawę funkcji mierzoną kwestionariuszem DASH o 22,11 ±16,52 pkt ($p \leq 0{,}001$) oraz PRTEE o 27,28 ±21,28 ($p \leq 0{,}001$). Powyższe wyniki odzwierciedla również subiektywna ocena stawu łokciowego (SEV) przez pacjentów, która wzrosła z 47,50 ±17,36 % do 73,77 ±21,04 % ($p \leq 0{,}001$).

W wyniku oceny zależności pomiędzy zawartością składników biologicznie aktywnych w PRP a skutecznością kliniczną leczenia iniekcyjnego entezopatii nadkłykcia bocznego kości ramiennej stwierdzono występowanie kilku istotnych statystycznie korelacji. Stopień zmniejszenia natężenia dolegliwości bólowych po trzech miesiącach od iniekcji dodatnio, na poziomie umiarkowanym, korelowała z zawartością w PRP płytek krwi ($r = 0{,}56$; $p \leq 0{,}001$), EGF ($r = 0{,}51$; $p < 0{,}05$), a także na słabym poziomie z zawartością VEGF ($r = 0{,}36$; $p < 0{,}05$), PDGF-AA ($r = 0{,}37$; $p < 0{,}05$) oraz PDGF-BB ($r = 0{,}44$; $p < 0{,}05$). Nie stwierdzono istotnych korelacji pomiędzy zawartością cytokin zapalnych w PRP a zmniejszeniem natężenia dolegliwości bólowych. Stopień poprawy siły uścisku oraz siły mięśniowej w zakresie prostowania nadgarstka dodatnio, aczkolwiek na poziomie słabym, korelowały z zawartością EGF w PRP (kolejno, $r = 0{,}44$, $r = 0{,}41$; $p < 0{,}05$), podobnie jak stopień poprawy funkcjonalnej mierzonej kwestionariuszem PRTEE ($r = 0{,}37$; $p < 0{,}05$). Interesującym wynikiem było również stężenie niektórych cytokin zapalnych (IL-10, IL-33, IL-17A) ujemnie korelujące z poprawą uzyskaną w kwestionariuszu PRTEE (kolejno, $r = -0{,}37$, $r = -0{,}44$, $-0{,}37$; $p < 0{,}05$).

Powyższe omówienie zawiera najważniejsze wyniki przeprowadzonych badań wchodzących w skład niniejszej rozprawy doktorskiej. Ze szczegółowymi wynikami można zapoznać się we właściwych częściach publikacji załączonych w kolejnych rozdziałach.

WNIOSKI

Omawiane publikacje wchodzące w skład rozprawy doktorskiej pozwoliły na realizację postawionych celów badawczych. Uzyskane wyniki zwiększają w sposób istotny dotychczasową wiedzę na temat autologicznego osocza bogatopłytkowego oraz jego użycia w leczeniu iniekcyjnym entezopatii nadkłykcia bocznego kości ramiennej. Mogą one pomóc w ukierunkowaniu dalszych prac nad optymalizacją protokołów przygotowywania PRP a także w poprawieniu algorytmów leczenia pacjentów z „łokciem tenisisty". Do najważniejszych wniosków płynących z przeprowadzonych badań należą:

- Pomiędzy zbadanymi czterema różnymi komercyjnymi systemami do przygotowywania PRP występują istotne różnice w zawartości składników biologicznie aktywnych w produkcie końcowym, w efektywności wychwytu płytek oraz powtarzalności uzyskiwanych stężeń płytek krwi.

- Największą ilość istotnych statystycznie różnic w zawartości czynników wzrostu i cytokin zapalnych zaobserwowano pomiędzy protokołem Mini GPS III (pozwalającym na powtarzalne uzyskiwanie wysokich (~5 x) stężeń płytek krwi w PRP) a protokołem Arthrex ACP, za pomocą którego uzyskiwano zagęszczenia na stałym poziomie ~1,5 x.

- Występują istotne dodatnie korelacje pomiędzy zawartością płytek krwi w PRP a stężeniem w nim takich czynników wzrostu jak EGF, VEGF, PDGF-AA, PDGF-BB.

- Występują istotne statystycznie korelacje pomiędzy zawartością leukocytów w PRP a stężeniem takich cytokin jak EGF, VEGF, HGF, PDGF-AA, PDGF-BB, IL-1β, MCP-1, IL-18, IL-8.

- PRP i surowica pochodzące od tego samego dawcy różnią się stężeniem poszczególnych składników aktywnych biologicznie jak również ich wzajemnymi proporcjami.

- W populacji męskiej w porównaniu do kobiecej zaobserwowano w PRP istotnie wyższą zawartość leukocytów oraz większości badanych cytokin zapalnych.

- Wiek oraz BMI nie miały istotnego wpływu na zawartość komórkową oraz czynników wzrostu w PRP.

- Występują istotne dodatnie korelacje pomiędzy zawartością w PRP płytek krwi, EGF, VEGF, PDGF-AA, PDGF-BB a stopniem redukcji dolegliwości bólowych pacjentów w 3 miesiącu po iniekcyjnym leczeniu entezopatii nadkłykcia bocznego kości ramiennej.

- Występuje istotna dodatnia korelacja pomiędzy zawartością EGF w PRP a stopniem poprawy siły uścisku, siły mięśniowej prostowników nadgarstka oraz poprawy funkcjonalnej mierzonej dedykowanym kwestionariuszem PRTEE u pacjentów w 3 miesiącu po iniekcyjnym leczeniu entezopatii nadkłykcia bocznego kości ramiennej.

- Występuje istotna ujemna korelacja pomiędzy zawartością IL-10, IL-33 oraz IL-17A w PRP a stopniem poprawy funkcjonalnej mierzonej dedykowanym kwestionariuszem PRTEE u pacjentów w 3 miesiącu po iniekcyjnym leczeniu entezopatii nadkłykcia bocznego kości ramiennej.

- Leczenie pacjentów z entezopatią nadkłykcia bocznego kości ramiennej poprzez lokalną iniekcję PRP jest skuteczną i bezpieczną metodą terapeutyczną.

BIBLIOGRAFIA

[1] Degen, R.M.; Conti, M.S.; Camp, C.L.; Altchek, D.W.; Dines, J.S.; Werner, B.C. Epidemiology and Disease Burden of Lateral Epicondylitis in the USA: Analysis of 85,318 Patients. *HSS J. Musculoskelet. J. Hosp. Spéc. Surg*. **2018**, 14, 9–14.

[2] Haahr, J.P. Prognostic factors in lateral epicondylitis: A randomized trial with one-year follow-up in 266 new cases treated with minimal occupational intervention or the usual approach in general practice. *Rheumatology* **2003**, 42, 1216–1225.

[3] Kraushaar, B.S.; Nirschl, R.P. Tendinosis of the Elbow (Tennis Elbow). Clinical features and findings of histological, immunohistochemical, and electron microscopy studies. *J. Bone Jt. Surg*. **1999**, 81, 259–278.

[4] Lian, J.; Mohamadi, A.; Chan, J.J.; Hanna, P.; Hemmati, D.; Lechtig, A.; Nazarian, A. Comparative Efficacy and Safety of Nonsurgical Treatment Options for Enthesopathy of the Extensor Carpi Radialis Brevis A Systematic Review and Meta-analysis of Randomized Placebo-Controlled Trials. *Am. J. Sports Med*. **2018**, 47, 3019–3029.

[5] Coombes, B.K.; Bisset, L.; Vicenzino, B. Efficacy and safety of corticosteroid injections and other injections for management of tendinopathy: A systematic review of randomised controlled trials. *Lancet* **2010**, 376, 1751–1767.

[6] Dhurat, R.; Sukesh, M. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. J. Cutan. *Aesthet. Surg*. **2014**, 7, 189.

[7] Marx, R.E. Platelet-rich plasma (PRP): What is PRP and what is not PRP? *Implant Dent.* **2001**, 10, 225–228.

[8] Mazzucco, L.; Balbo, V.; Cattana, E.; Guaschino, R.; Borzini, P. Not every PRP-gel is born equal Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet®, RegenPRP-Kit®, Plateltex® and one manual procedure. *Vox Sang.* **2009**, 97, 110–118.

[9] Werner, S.; Grose, R. Regulation of wound healing by growth factors and cytokines. *Physiol. Rev*. **2003**, 83, 835–870.

[10] Kobayashi, Y.; Saita, Y.; Nishio, H.; Ikeda, H.; Takazawa, Y.; Nagao, M.; Takaku, T.; Komatsu, N.; Kaneko, K. Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations. *J. Orthop. Sci*. **2016**, 21, 683–689

[11] Muthu, S.; Patel, S.; Selvaraj, P.; Jeyaraman, M. Comparative analysis of leucocyte poor vs. leucocyte rich platelet-rich plasma in the management of lateral epicondylitis: Systematic review & meta-analysis of randomised controlled trials. *J. Clin. Orthop. Trauma.* **2021**, 19, 96–107.

[12] Hussain, N.; Johal, H.; Bhandari, M. An evidence-based evaluation on the use of platelet rich plasma in orthopedics - a review of the literature. *Sicot-J.* **2017**, 3, 57.

[13] Simental-Mendía, M.; Vilchez-Cavazos, F.; Álvarez-Villalobos, N.; Blázquez-Saldaña, J.; Peña-Martínez, V.; Villarreal-Villarreal, G.; Acosta-Olivo, C. Clinical efficacy of platelet-rich plasma in the treatment of lateral epicondylitis: A systematic review and meta-analysis of randomized placebo-controlled clinical trials. *Clin. Rheumatol*. **2020**, 39, 2255–2265.

[14] de Vos, R.-J.; Windt, J.; Weir, A. Strong evidence against platelet-rich plasma injections for chronic lateral epicondylar tendinopathy: A systematic review. *Br. J. Sports Med*. **2014**, 48, 952–956.

[15] Oudelaar, B.W.; Peerbooms, J.C.; Huis in 't Veld, R.; Vochteloo, A.J.H. Concentrations of Blood Components in Commercial Platelet-Rich Plasma Separation Systems: A Review of the Literature. Am. *J. Sports Med*. **2019**, 47, 479–487.

[16] Dohan Ehrenfest, D.M.; Rasmusson, L.; Albrektsson, T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. **2009**, 27, 158–167.

[17] Lana, J.F.S.D.; Purita, J.; Paulus, C.; Huber, S.C.; Rodrigues, B.L.; Rodrigues, A.A.; Santana, M.H.; Madureira, J.L.; Malheiros Luzo, Â.C.; Belangero, W.D.; et al. Contributions for classification of platelet rich plasma—Proposal of a new classification: MARSPILL. *Regen. Med*. **2017**, 12, 565–574.

[18] Kushida, S.; Kakudo, N.; Morimoto, N.; Hara, T.; Ogawa, T.; Mitsui, T.; Kusumoto, K. Platelet and growth factor concentrations in activated platelet-rich plasma: a comparison of seven commercial separation systems. *J Artif Organs* **2014**; 17: 186-192.

[19] Dejnek, M.; Moreira, H.; Płaczkowska, S.; Morasiewicz, P.; Barg, E.; Witkowski, J.; Reichert, P. Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study. *Adv. Clin. Exp. Med*. **2021**, 30, 757–764.

[20] Dejnek, M.; Witkowski, J.; Moreira, H.; Płaczkowska, S.; Morasiewicz, P.; Reichert, P.; Królikowska, A. Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods. *World J Orthop.* **2022**, 13(6), 587-602.

[21] Dejnek, M.; Moreira, H.; Płaczkowska, S.; Barg, E.; Reichert, P.; Królikowska, A. Leukocyte-Rich Platelet-Rich Plasma as an Effective Source of Molecules That Modulate Local Immune and Inflammatory Cell Responses. *Oxid Med Cell Longev*. **2022**, 2022:8059622.

[22] Dejnek, M.; Moreira, H.; Płaczkowska, S.; Barg, E.; Reichert, P.; Królikowska, A. Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma. *J. Clin. Med.* **2022**, 11, 3687.

[23] Lim, W.; Park, S.H.; Kim, B.; Kang, S.W.; Lee, J.W.; Moon, Y.L. Relationship of cytokine levels and clinical effect on platelet-rich plasma-treated lateral epicondylitis. *J. Orthop. Res*. **2018**, 36, 913–920.

[24] Kim, S.J.; Yeo, S.M.; Noh, S.J.; Ha, C.-W.; Lee, B.C.; Lee, H.S.; Kim, S.J. Effect of platelet-rich plasma on the degenerative rotator cuff tendinopathy according to the compositions. *J. Orthop. Surg. Res*. **2019**, 14, 408.

[25] Louis, M.L.; Magalon, J.; Jouve, E.; Bornet, C.E.; Mattei, J.C.; Chagnaud, C.; Rochwerger, A.; Veran, J.; Sabatier, F. Growth Factors Levels Determine Efficacy of Platelets Rich Plasma Injection in Knee Osteoarthritis: A Randomized Double Blind Noninferiority Trial Compared with Viscosupplementation. Arthrosc. *J. Arthrosc. Relat. Surg*. **2018**, 34, 1530–1540.

III. PRACA NR 1:

Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study

Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study

Maciej Dejnek[1,2,A–F], Helena Moreira[3,A–F], Sylwia Płaczkowska[4,B], Piotr Morasiewicz[5,A,F], Ewa Barg[3,A,E], Jarosław Witkowski[1,2,D,E], Paweł Reichert[1,A,E,F]

[1] Department of Trauma and Hand Surgery, Wroclaw Medical University, Poland
[2] Department of Sports Medicine, Wroclaw Medical University, Poland
[3] Department of Basic Medical Sciences, Wroclaw Medical University, Poland
[4] Diagnostic Laboratory for Teaching and Research, Department of Laboratory Diagnostics, Wroclaw Medical University, Poland
[5] Department of Orthopaedic and Trauma Surgery, Institute of Medical Sciences, University of Opole, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Advances in Clinical and Experimental Medicine, ISSN 1899–5276 (print), ISSN 2451–2680 (online) *Adv Clin Exp Med*. 2021;30(7):757–764

Address for correspondence
Maciej Dejnek
E-mail: maciejdejnek@gmail.com

Funding sources
This research was financially supported by the Ministry of Health subvention according to number of STM.E067.20.112 from the IT Simple system of Wroclaw Medical University.

Conflict of interest
None declared

Received on December 30, 2020
Reviewed on February 6, 2021
Accepted on March 26, 2021

Published online on June 11, 2021

Cite as
Dejnek M, Moreira H, Płaczkowska S, et al. Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study. *Adv Clin Exp Med*. 2021;30(7):757–764. doi:10.17219/acem/135045

DOI
10.17219/acem/135045

Copyright
Copyright by Author(s)
This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported (CC BY 3.0) (https://creativecommons.org/licenses/by/3.0/)

Abstract

Background. Autologous platelet-rich plasma (PRP) injection is an alternative but widely accepted method for the treatment of degenerative changes in tendon attachments known as enthesopathies. The PRP is considered a safe source for high concentrations of the growth factors involved in the healing process. Despite initial promising outcomes, many recent studies report conflicting results for this treatment. This may be due to differences in the concentrations of platelets and growth factors in PRPs obtained using different methods.

Objectives. The aim of this study was to compare PRP preparation systems in terms of morphotic components and selected growth factors to find the most appropriate procedure for the treatment of enthesopathies.

Materials and methods. Whole blood samples from 6 healthy male volunteers were collected. Using different commercial kits (Mini GPS III System, Arthrex ACP, and Xerthra, Dr. PRP), 4 PRPs were prepared from the blood of each participant. All samples were analyzed for the content of morphotic components and the following growth factors: transforming growth factor-β1 (TGF-β1), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor AA (PDGF-AA).

Results. The Mini GPS III produced PRP with the highest concentration of platelets and white blood cells (WBC) compared to the other systems included in the study. Significant differences in the levels of EGF and PDGF-AA were found only between the Mini GPS III and Arthrex ACP. There was positive correlation between the content of platelets and the levels of PDGF-AA and EGF. The red blood cells (RBC) concentration positively correlated with PDGF-AA, EGF and VEGF.

Conclusions. This study showed differences between the morphotic components and levels of selected growth factors in PRP obtained with the different preparation methods. Due to insufficient data, we cannot argue for or against any of the studied protocols for the treatment of enthesopathy. Further studies on a larger population are required to validate our results.

Key words: platelet-rich plasma, growth factors, platelet-derived growth factor, enthesopathy

Background

Enthesopathies are degenerative changes in the site of tendon attachment to the bone. This disease can affect many different sites in the human body. The most common and best described are changes in the attachment of wrist extensors or flexors to humeral epicondyles, the Achilles tendon, patellar tendon, suprascapular tendon, or plantar fascia.[1] The main symptoms reported by patients include local pain and limitations in sport, work and daily activities. The cause of the disease still remains unclear. However, the most widely accepted theory is the accumulation of microinjuries resulting from repeated overloads that exceed the body's compensatory capacity. The change in the dogma of the inflammatory nature of this disease was the result of numerous histopathological studies that found disorganized tissue and neovessels within the involved tendon, but only few inflammatory cells. In many cases, symptoms resolve spontaneously and properly selected exercises help prevent them in the future. Unfortunately, some cases turn into a chronic condition that is very difficult to treat, and sometimes only surgical excision of the affected tissue can lead to improvement.

Due to the lack of effective therapy, many different methods have been proposed, and autologous platelet-rich plasma (PRP) is one of the most promising treatments.[1–3] For this procedure, the patient's blood is collected and centrifuged to isolate the platelet-rich part of the plasma. This plasma is then administered into the affected tissue, typically by local injection. The α-granules of platelets contain significant amounts of cytokines involved in tissue healing.[4] It is expected that PRP containing a platelet concentration above the baseline will contain a significantly higher concentration of important cytokines, chemokines and growth factors.[5] Numerous in vitro studies have shown that these biologically active components play a key role in tissue repair by stimulating proliferation, chemotaxis, cell differentiation, and angiogenesis.[4,6,7]

The acceleration of natural tissue healing processes by PRP administration was expected to revolutionize the treatment of injuries and chronic degenerative diseases like enthesopathy. Other diseases in which the natural balance between anabolic and catabolic processes is disrupted are also candidates for PRP treatment. Positive clinical outcomes have been reported for various conditions including bone nonunions, osteonecrosis, difficult-to-heal wounds, osteoarthritis, and sports injuries.[8–12]

The enthusiastic adoption of this method began to wane with the increasing appearance of studies showing conflicting results.[12–14] A major problem with this body of research is the lack of a standardized definition for PRP. Many manufacturers have released commercially available kits for the easy preparation of PRP in an outpatient setting.[8,15] These kits differ from each other in various parameters, such as the amount of material collected from the patient, the type of anticoagulant used, the structure of the separator, the length and speed of centrifugation, the method of extraction and activation, the assumed concentration of platelets and leukocytes in final product, and its consistency.[16–19] These differences have made it almost impossible to compare the results of studies where PRP was prepared according to different protocols.

The answer to the above problem should be a reliable classification system for PRP. One of the classification systems designed to standardize the nomenclature is based on the content of fibrin and leukocytes in the PRP. In this system, 4 main classes of autologous PRPs are identified — PRP with a low content of leukocytes (P-PRP), PRP with a high content of leukocytes (L-PRP), platelet-rich fibrin with a low content of leukocytes (P-PRF), and platelet-rich fibrin with a high content of leukocytes (L-PRF).[20] However, these divisions do not include the concentration or the absolute number of platelets. Another classification system proposed to improve the comparison of results from different publications is the PAW system. This system is based on the 3 most important components of PRP: the absolute number of platelets, the method of their activation and the leukocyte content.[21]

The classification systems mentioned above do not solve all the problems connected with studies on PRP. The multiplicity of variables still makes it almost impossible to predict the content of different growth factors in PRP.

Objectives

The main goal of this study is to compare the PRP preparation systems available on the local market in terms of morphotic components and selected growth factors.

The results of such an analysis are essential for selecting the most appropriate procedure for daily clinical practice and for further research on the treatment of degenerative conditions. Regarding legal issues, only those systems that are officially registered for the treatment of enthesopathy were included in the study. However, our results can be helpful in decision-making for all conditions treated with PRP.

Materials and methods

Study design and setting

This controlled laboratory study was conducted at Wroclaw Medical University, Poland, in the Diagnostic Laboratory for Teaching and Research by clinicians and laboratory researchers. All procedures on human participants were conducted in accordance with the ethical standards of Wroclaw Medical University (Poland) and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the local bioethics committee (Ethics Committee of Wroclaw Medical University, 30.03.2020, approval No. KB 163/2020).

Participants

Six healthy male volunteers similar in age were asked for a whole blood donation. The number of participants included reflects the preliminary nature of the study. Criteria for inclusion in the study were an age of 27–28 years, absence of significant disease and conditions that could affect the blood morphotic components, at least 2 weeks without taking any drug that may interfere with the function of platelets, and a non-smoking status.

Data sources and measurement

Approximately 75 mL of whole blood was collected from each participant under aseptic conditions and immediately divided into 5 samples. First, ~2 mL was transferred to a tube with ethylenediaminetetraacetic acid (EDTA) and then analyzed for morphotic components using an automatic laboratory analyzer (Mindray BC-5150; Shenzhen Mindray Bio-Medical Electronics Co., Ltd. Shenzen, China). The 4 remaining blood samples from each participant were used to prepare 4 different PRPs according to the protocols provided by the manufacturers. Four commercial PRP systems that were available on the local medical market were chosen as they are frequently used for the treatment of orthopedic conditions. The systems used included the Arthrex Autologous Conditioned Plasma (ACP) Double Syringe system (Arthrex Inc., Naples, USA), the Mini GPS III Platelet Concentration system (Biomet Inc., Warsaw, USA), the Xerthra PRP kit (Biovico Sp. z o.o., Gdynia, Poland), and Dr. PRP (Rmedica, Seoul, South Korea).

The Arthrex ACP Double Syringe system required 13.5 mL of whole blood collected into a specially designed double-syringe system within 1.5 mL ACD-A added as an anticoagulant. The samples were spun at 1500 rpm for 5 min in a dedicated centrifuge provided by the local distributor. After centrifugation, conditioned plasma in a volume of 4 mL was transferred to the inner syringe in the double-syringe system and was ready to use.

The Mini GPS III Platelet Concentration system has a specially designed valve for automatic PRP separation. After mixing 27 mL of whole blood with 3 mL of ACD-A anticoagulant, the samples were placed in a separator and spun at 3200 rpm for 15 min in a dedicated centrifuge provided by the local distributor. The platelet-poor plasma was then removed and about 3 mL of leukocyte-rich (LR)-PRP was collected into a new sterile syringe.

The Xerthra PRP kit required 13.5 mL of whole blood that was mixed with 1.5 mL of 3.13% sodium citrate as an anticoagulant. The samples were spun at 3500 rpm for 5 min in a dedicated centrifuge provided by the local distributor, transferring plasma into the neck of the tube and removing platelet poor plasma 1.5 mL of leukocyte-poor (LP)-PRP was then collected into a new sterile syringe.

Whole blood in a volume of 18 mL mixed with 2 mL of 3.13% sodium citrate was transferred into the Dr. PRP tube. The samples were then spun for 4 min at 3100 rpm in a dedicated centrifuge provided by the local distributor. Following this, the piston in the device was used to separate the plasma from the red blood cells (RBC) by moving it into the neck of the tube. After removing platelet poor plasma, LP-PRP in a volume of 3 mL was collected into a new sterile syringe.

All 24 samples of prepared plasma were analyzed for the content of morphotic components using an automatic laboratory analyzer (Mindray BC-5150). The count and concentration of white blood cells (WBC), RBC and platelets were evaluated immediately after collection of the samples. Platelet capture efficiency (PCE) was calculated using the following formula:

obtained PRP volume [mL] × platelets concentration in PRP (G/L)/whole blood collected volume [mL] × platelets concentration in whole blood (G/L).

Following collection, 1 mL of each PRP sample was placed into an Eppendorf polypropylene tube and went through the platelet activation process developed by Zimmermann et al.[22] The activation procedure involved freezing at −80°C for 30 min, followed by thawing to room temperature for another 30 min and freezing for a second time at −80°C. The samples were then stored at −80°C until further analysis.

Before cytokine measurement, the PRP samples were thawed completely at room temperature and spun for 5 min at 2.5 rpm using a Micro Star 17 centrifuge (VWR International Company, ThermoElectron LED, Langenselbold, Germany). A custom-designed bead-based multiplex immunoassay that uses fluorescence-encoded beads and flow cytometry (LEGENDplexTM; BioLegend, San Diego, USA) was used to quantify the following platelet growth factors: transforming growth factor-β1 (TGF-β1, free active), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor-AA (PDGF-AA). The concentration of a particular cytokine was determined by means of a standard curve generated during the performance of the test. The analyses were done according to the manufacturer's instructions. The samples were acquired on CyFlow SPACE and a CyFlow CUBE flow cytometer (Sysmex-Partec, Görlitz, Germany) by applying a 488 nm laser with a 536/40 (BP) filter for the PE fluorochrome, and a 638 nm laser with a 675/20 (BP) filter for the APC fluorochrome. The results were analyzed with LEGENDplexTM Data Analysis Software v. 8.0 (Vigene Tech Inc., Carlisle, USA).

All data obtained and analyzed in this study are quantitative. To avoid bias in the obtained results, all PRP samples were made with the same great care.

Statistical methods

All data were analyzed using STATISTICA v. 13.3 software (StatSoft Inc., Tulsa, USA). Due to the lack of confirmation of to a normal distribution, as assessed with

the Shapiro–Wilk test, nonparametric methods were used (Kruskal–Wallis one-way analysis of variance (ANOVA) with Dunn's post hoc test and Spearman's correlation). Statistical significance was established at the level of $p < 0.05$.

Results

Study participants

Six volunteers, aged 27.8 ±0.4 years, met the criteria for inclusion and were enrolled in the study. The average body mass index (BMI) of the volunteers was 25.82 ±2.12 kg/m². Each participant signed an informed consent form.

Main results

Whole blood count

The distribution of the cellular components of whole blood samples collected from all participants are included in Table 1. All results were in the range of normal physiological values.

Concentration of platelets

The platelet concentrations for the PRP samples are illustrated in Fig. 1. Only 1 single sample of PRP delivered by Xerthra PRP kit had a lower concentration of platelets than baseline. The highest platelet concentration was produced with the Mini GPS III System (1266.33 ±347.96 × 10^9/L), and the lowest with Arthrex ACP (395 ±110.15 × 10^9/L). Statistical analysis showed a significant difference between the 2 systems mentioned above ($p = 0.003$), and between the Mini GPS III and Xerthra PRP kits (513.67 ±255.99 × 10^9/L; $p = 0.04$). There was no significant difference when compared to Dr. PRP (504.83 ±106.29 × 10^9/L; $p > 0.05$).

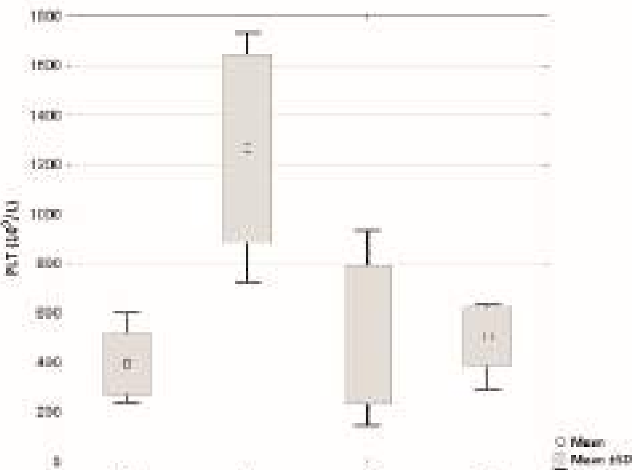


Fig. 1. Platelet concentration in PRP obtained using different systems

A – Arthrex ACP; B – Mini GPS III System; C – Xerthra PRP kit; D – Dr. PRP

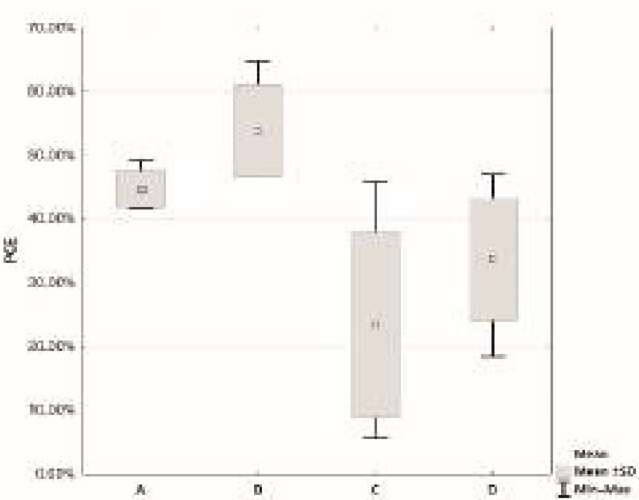


Fig. 2. Platelet capture efficiency in PRP obtained using different systems

A – Arthrex ACP; B – Mini GPS III System; C – Xerthra PRP kit; D – Dr. PRP

PCE

The PCE results are illustrated in Fig. 2. The highest PCE score was obtained from the Mini GPS III system (53.76 ±6.66%), and the lowest from the Xerthra PRP kit (23.50 ±13.13%). The Mini GPS III System provided a significantly higher PCE then the Xerthra PRP kit ($p = 0.001$) and Dr. PRP (33.68 ±8.78%; $p = 0.02$). Although the PCE

Table 1. Whole blood characteristics from all participants (NR 1–6)

Blood parameter	Participant						Mean
	NR1	NR2	NR3	NR4	NR5	NR6	
RBC [10^{12}/L]	4.69	4.47	4.97	5.82	5.5	5.04	5.08 ±0.46
Platelets [10^9/L]	171	288	244	226	364	264	259.5 ±59.09
WBC [10^9/L]	4.79	5.62	7.22	6.5	7.64	6.19	6.33 ±0.95
Neutrophils [10^9/L]	2.52	3.01	4.13	3.34	4.37	2.97	3.39 ±0.66
Lymphocytes [10^9/L]	1.79	1.83	2.41	2.44	2.53	2.53	2.26 ±0.32
Monocytes [10^9/L]	0.34	0.4	0.53	0.49	0.54	0.45	0.46 ±0.07
Eosinophils [10^9/L]	0.11	0.34	0.11	0.18	0.17	0.21	0.19 ±0.08
Basophils [10^9/L]	0.03	0.04	0.04	0.05	0.03	0.03	0.04 ±0.01

RBC – red blood cells; WBC – white blood cells.

obtained from the Arthrex ACP kit (44.66 ±2.65%) was lower than that for the Mini GPS III and higher than that for the Xerthra PRP and Dr. PRP kits, the differences were not statistically significant ($p = 0.061$, $p = 0.22$, $p = 1$, respectively).

Concentration of WBC

The WBC concentrations for the PRP samples are illustrated in Fig. 3. The highest concentrations of WBC were obtained with the Mini GPS III system ($34.81 \pm 9.59 \times 10^9$/L), and it was the only system that produced a WBC concentration above the whole blood baseline level. Statistical analysis showed significant differences when comparing Mini GPS III to Arthrex ACP ($0.78 \pm 0.73 \times 10^9$/L; $p = 0.02$) and Dr. PRP ($0.50 \pm 0.59 \times 10^9$/L; $p = 0.001$), but not to the Xerthra PRP kit ($1.91 \pm 1.87 \times 10^9$/L; $p = 0.16$).

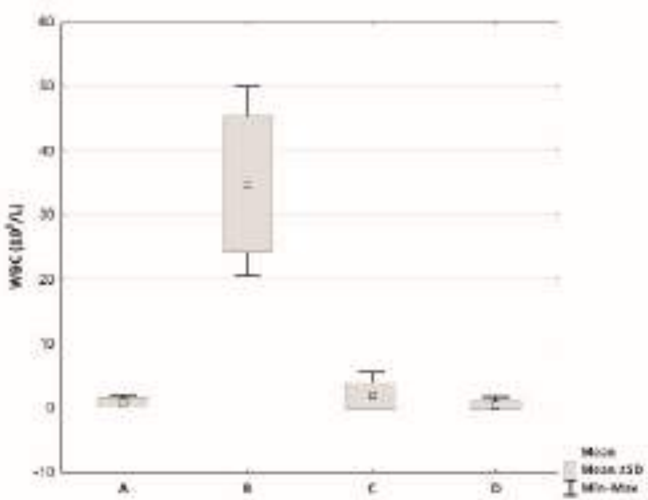


Fig. 3. White blood cells concentration in PRP obtained using different systems

A – Arthrex ACP; B – Mini GPS III System; C – Xerthra PRP kit; D – Dr. PRP

Concentration of RBC

The RBC concentrations for the PRP samples are illustrated in Fig. 4. One of the goals of producing PRP is to separate it from plasma containing RBC. Hence, the RBC concentration in PRP can be treated as a measure of purification. The Mini GPS III System delivered PRP with the highest concentration of RBC ($1.48 \pm 0.88 \times 10^{12}$/L), which was significantly higher than

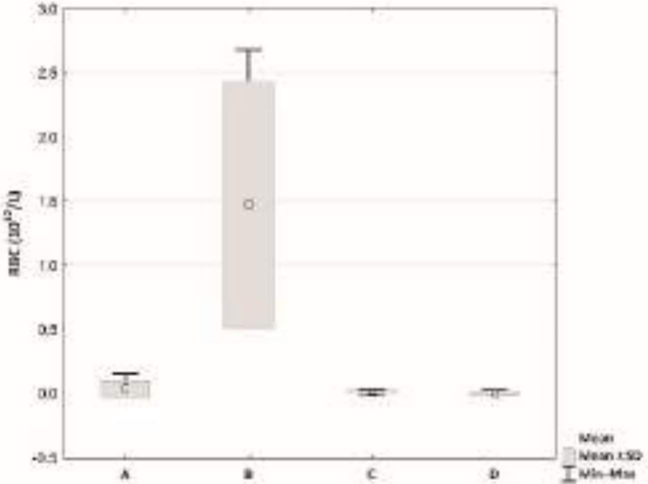


Fig. 4. Red blood cells concentration in PRP obtained using different systems

A – Arthrex ACP; B – Mini GPS III System; C – Xerthra PRP kit; D – Dr. PRP.

Arthrex ACP ($0.04 \pm 0.06 \times 10^{12}$/L; $p = 0.045$), the Xerthra PRP kit ($0.02 \pm 0.01 \times 10^{12}$/L; $p = 0.04$) and Dr. PRP ($0.01 \pm 0.01 \times 10^{12}$/L; $p = 0.004$). No significant differences were found between the other systems.

Concentration of growth factors

All obtained growth factor concentrations are included in Table 2. Statistical analysis showed that the Mini GPS III System compared to Arthrex ACP delivered PRP with significantly higher levels of EGF (364.1 ±180.16 pg/mL compared to 107.37 ±95.12 pg/mL; $p = 0.04$) and PDGF-AA (98,698 ±23,843.58 pg/mL compared to 33,172.5 ±13,266.38 pg/mL; $p = 0.02$). There were no significant differences among the other systems and growth factors.

Correlation between growth factors and morphotic components

All Spearman's correlations are presented in Fig. 5. There was a significant positive correlation between platelet concentration and both EGF and PDFG-AA (Spearman's R values 0.46 and 0.58, respectively). A significant positive correlation was also observed between the WBC concentration and PDFG-AA (Spearman's R value 0.51). The RBC concentration was also significantly positively correlated

Table 2. Concentration of growth factors in PRP obtained using different systems

System	Growth factor			
	TGF-β1 [pg/mL]	EGF [pg/mL]	VEGF [pg/mL]	PDGF-AA [pg/mL]
Arthrex ACP	58.12 ±76.92	107.37 ±95.12	138.88 ±189.52	33172.5 ±13266.38
Mini GPS III System	31.72 ±17.26	364.1 ±180.16	456.06 ±301.51	98698 ±23843.58
Xerthra PRP kit	45.97 ±59.60	161.20 ±125.34	288.61 ±364.54	54565.43 ±43241.12
Dr. PRP	30.18 ±25	223.48 ±173.63	187.58 ±134.90	41400.6 ±18537.8

TGF-β1 – transforming growth factor-β1; EGF – epidermal growth factor; VEGF – vascular endothelial growth factor; PDGF-AA – platelet-derived growth factor-AA.

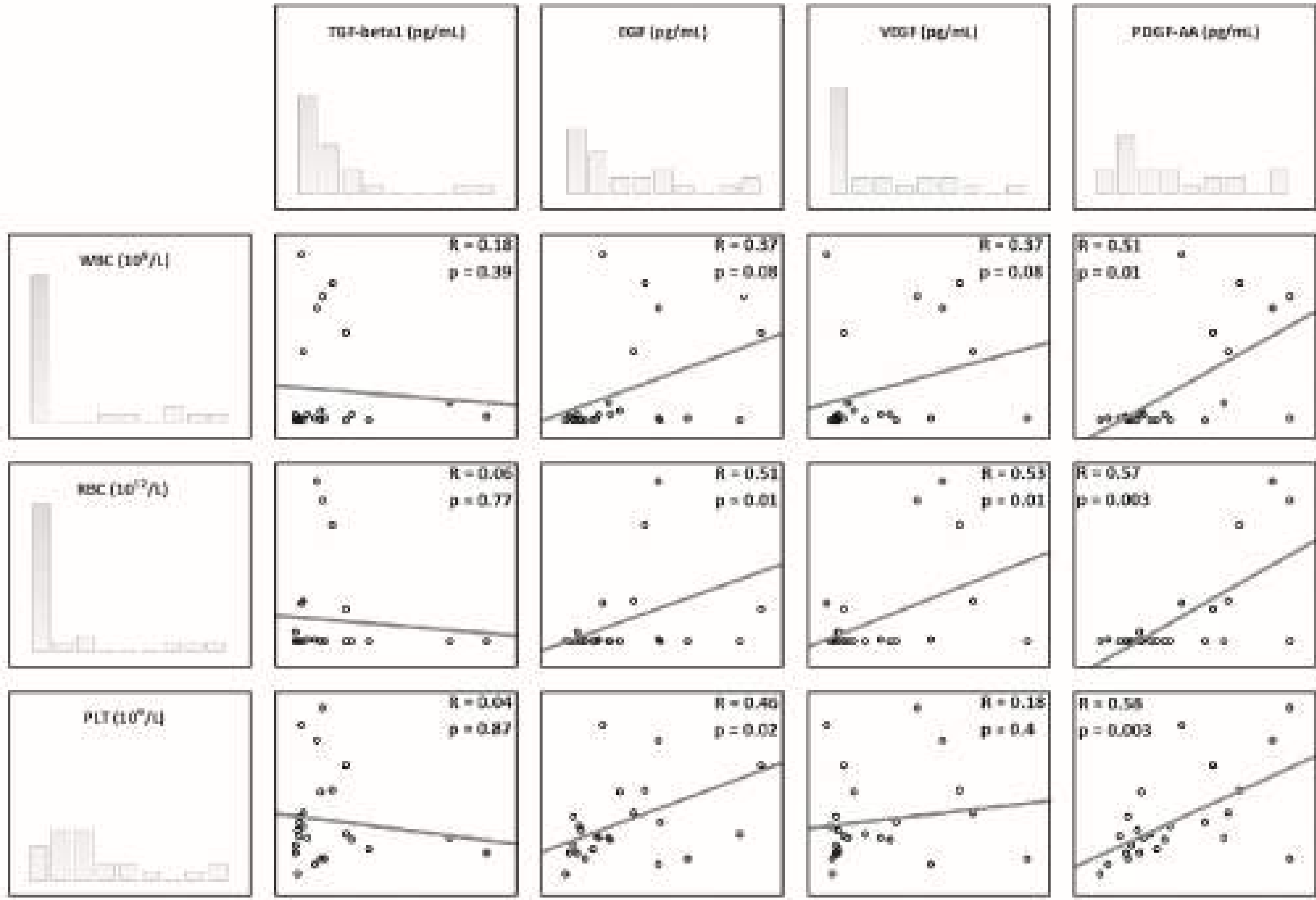


Fig. 5. Spearman correlations between morphotic components and growth factors in PRP samples

with the levels of EGF, VEGF and PDGF-AA (Spearman's R values 0.51, 0.53 and 0.57, respectively). No significant correlation was found between TGF-β1 and any PRP morphotic component.

Discussion

As expected, the current study showed differences between PRP produced with the use of various commercial kits. These findings confirm the results of other studies that have been summarized in a recent systemic review.[19] However, it is important to point out that these differences do not determine the possible clinical superiority of any of the described systems.

All kits used in the current study produced PRP with an average platelet concentration above the whole blood baseline level. For unexplained reasons, 1 PRP sample obtained using the Xerthra PRP system had a platelet concentration below the baseline level. The Mini GPS III System produced the highest platelet concentration in relation to the whole blood baseline level (×4.84 ±0.6), while the lowest concentration was generated with the Arthrex ACP system (×1.51 ±0.09). These 2 systems are among the most studied and others have reported similar results for platelet concentrations.[17,19,23,24] The Xerthra PRP kit and Dr. PRP were able to concentrate platelets in similar manner (×2.11 ±1.18 and ×2.02 ±0.53, respectively).

Both too low and too high a platelet concentration can reduce the chances of a good therapeutic response. Platelet levels below the baseline are not sufficient to induce a significant response and a concentration above ×6 may slow down the repair processes.[21] Graziani et al. concluded that platelet concentrations of approx. 2.5 times greater than native blood achieved a maximum effect on osteoblast and fibroblast proliferation in vitro. In addition, higher dosages 3.5 times above baseline could lead to some adverse events.[25] Various studies have also reported different ideal therapeutic platelet concentrations. While some authors recommend a platelet concentration of about 1000×10^3/mL, others consider a number $>200 \times 10^3$/mL as sufficient.[26,27] According to this latter definition of PRP, all samples included in this study met the criteria.[28]

In the current study, there were significant correlations observed between platelet concentration and both EGF and PDGF-AA, but no correlations with VEGF and TGF-β1. These results are somewhat different from what has been reported previously. For example, Magalon observed significant correlations between platelet dose and all growth factors examined (VEGF, EGF, PDGF-AB, and

TGF-β1).[17] Similarly, Sundman also reported positive significant correlations between platelets and both TGF-β1 and PDGF-AB.[29]

Significantly higher concentrations of WBC were delivered by the Mini GPS III system as it is designed for LR-PRP production. The mean concentration of WBC produced by this system was 5.55 ±1.65 times above the baseline in whole blood. The mean content of neutrophils and leukocytes were distributed almost equally (15.72 ±8.11 × 10^9/L and 15.99 ±2.96 × 10^9/L, respectively). Similar results have been observed in previous studies.[17–19,23,24,29] Arthrex ACP, Xerthra PRP kit and Dr. PRP delivered LP-PRP with a WBC concentration much lower than the baseline level. According to the literature, the presence of leukocytes in PRP could affect the levels of important growth factors such as VEGF and EGF, and may also have antibacterial or immune-regulating effects.[17,18,30] While we did not observe a correlation between WBC and both VEGF and EGF, a positive correlation with PDGF-AA was found. This finding has not been reported in previous research, likely because PDGF-AA is not a frequently analyzed cytokine. In vitro studies have shown a potential negative effect on the healing of tendon structures due to the high content of proteinases and hydrolases in WBC, especially in neutrophils.[29,31–33] In vivo studies do not confirm this effect; however, when planning the therapy for enthesopathies, one should take into consideration the possible stimulation of catabolic processes by a high content of leukocytes.[15,21]

An efficient PRP preparation procedure should remove RBC as much as possible, as their presence is considered as the sign of impurity. This is likely the reason why the most studies do not analyze the correlation between RBC and growth factors. Our results showed a significant correlation between RBC concentration and levels of growth factors such as EGF, VEGF and PDGF-AA. The strength of correlation was moderate (Spearman's R value between 0.51 and 0.57) but still worthy of further analysis in a larger population. Among the tested PRP preparation kits, the Mini GPS III System produced a significantly higher RBC contamination compared to other systems. This may be one of the factors behind the higher growth factor content in PRP obtained by the Mini GPS III System in other studies.[17,19] Arthrex ACP, Xerthra PRP kit and Dr. PRP delivered PRP with an almost undetectable RBC concentration.

Limitations

To date, numerous studies have already shown the diversity in the morphotic components and growth factor content in PRP obtained various using methods. However, systems such as the Xerthra PRP kit and Dr. PRP were tested here for the first time. There are many more commercially available systems for PRP preparation than those included in the study. Even if we chose the most popular systems, it does not allow the results to be transferred to other systems, and they have to be evaluated separately.

As this is a preliminary study, we decided to examine only a few selected growth factors. Many other cytokines, chemokines and growth factors released by platelets are also involved in tissue healing. The task for future research is to study all of the cytokines that play a key role in regenerative processes. On the other hand, even a precise determination of the cytokine content does not allow one to predict clinical effects in vivo. Therefore, it will be necessary to evaluate how differences in PRP characteristics affect living tissues.

Due to the lack of confirmation to a normal distribution for most of the data, nonparametric methods were used for statistical analyses. This lack of confirmation was likely due to the small sample size used, and extension to a larger group of participants may provide more reliable results.

Conclusions

The current study showed a wide heterogeneity in the characteristics of autologous PRPs produced by various commercial kits. Based on the obtained results and previous studies, the correlation between the concentration of desired growth factors and morphotic components remains unclear. Due to insufficient data, we cannot argue for or against use of any of the mentioned protocols for the treatment of enthesopathy. Further studies on a larger population that examine a wider variety of cytokines are required to validate our results. Future research should also focus on both the in vitro and in vivo biological effects of PRP produced by different preparation protocols to establish the effects of different concentrations of the various growth factors on tissue healing.

ORCID iDs

Maciej Dejnek https://orcid.org/0000-0002-6675-0256
Helena Moreira https://orcid.org/0000-0002-8084-3686
Sylwia Płaczkowska https://orcid.org/0000-0002-1466-3820
Piotr Morasiewicz https://orcid.org/0000-0002-7587-666X
Ewa Barg https://orcid.org/0000-0002-7069-5026
Jarosław Witkowski https://orcid.org/0000-0002-2754-1339
Paweł Reichert https://orcid.org/0000-0002-0271-4950

References

1. Fitzpatrick J, Bulsara M, Zheng MH. The effectiveness of platelet-rich plasma in the treatment of tendinopathy. *Am J Sports Med*. 2017;45(1):226–233. doi:10.1177/0363546516643716
2. Mi B, Liu G, Zhou W, et al. Platelet rich plasma versus steroid on lateral epicondylitis: Meta-analysis of randomized clinical trials. *Phys Sportsmed*. 2017;45(2):97–104. doi:10.1080/00913847.2017.1297670
3. Królikowska A, Sikorski Ł, Czamara A, Reichert P. Are the knee extensor and flexor muscles isokinetic parameters affected by the duration of postoperative physiotherapy supervision in patients eight months after ACL reconstruction with the use of semitendinosus and gracilis tendons autograft? *Acta Bioeng Biomech*. 2018;20(4):89–100. doi:10.5277/ABB-01149-2018-02
4. Sánchez-González DJ, Méndez-Bolaina E, Trejo-Bahena NI. Platelet-rich plasma peptides: Key for regeneration. *Int J Pept*. 2012;2012:532519. doi:10.1155/2012/532519
5. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: The state of play. *Br J Sports Med*. 2008;42(5):314–320. doi:10.1136/bjsm.2007.040071

6. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev*. 2003;83(3):835–870. doi:10.1152/physrev.2003.83.3.835
7. Molloy T, Wang Y, Murrell GAC. The roles of growth factors in tendon and ligament healing. *Sport Med*. 2003;33(5):381–394. doi:10.2165/00007256-200333050-00004
8. Alsousou J, Thompson M, Hulley P, Noble A, Willett K. The biology of platelet-rich plasma and its application in trauma and orthopaedic surgery: A review of the literature. *J Bone Joint Surg Br*. 2009;91(8):987–996. doi:10.1302/0301-620X.91B8.22546
9. Tenore G, Zimbalatti A, Rocchetti F, et al. Management of medication-related osteonecrosis of the kaw (MRONJ) using leukocyte- and platelet-rich fibrin (L-PRF) and photobiomodulation: A retrospective study. *J Clin Med*. 2020;9(11):3505. doi:10.3390/jcm9113505
10. Suthar M, Gupta S, Bukhari S, Ponemone V. Treatment of chronic non-healing ulcers using autologous platelet rich plasma: A case series. *J Biomed Sci*. 2017;24(1):16. doi:10.1186/s12929-017-0324-1
11. Tietze DC, Geissler K, Borchers J. The effects of platelet-rich plasma in the treatment of large-joint osteoarthritis: A systematic review. *Phys Sportsmed*. 2014;42(2):27–37. doi:10.3810/psm.2014.05.2055
12. Moraes VY, Lenza M, Tamaoki MJ, Faloppa F, Belloti JC. Platelet-rich therapies for musculoskeletal soft tissue injuries. *Cochrane Database Syst Rev*. 2013;12:CD010071. doi:10.1002/14651858.CD010071.pub2
13. Scott A, LaPrade RF, Harmon KG, et al. Platelet-rich plasma for patellar tendinopathy: A randomized controlled trial of leukocyte-rich PRP or leukocyte-poor PRP versus saline. *Am J Sports Med*. 2019;47(7):1654–1661. doi:10.1177/0363546519837954
14. Navani A, Li G, Chrystal J. Platelet rich plasma in musculoskeletal pathology: A necessary rescue or a lost cause? *Pain Physician*. 2017;20(3):E345–E356. doi:10.36076/ppj.2017.e356
15. Wasterlain AS, Braun HJ, Dragoo JL. Contents and formulations of platelet-rich plasma. *Oper Tech Orthop*. 2012;22(1):33–42. doi:10.1053/j.oto.2011.11.001
16. Kushida S, Kakudo N, Morimoto N, et al. Platelet and growth factor concentrations in activated platelet-rich plasma: A comparison of seven commercial separation systems. *J Artif Organs*. 2014;17(2):186–192. doi:10.1007/s10047-014-0761-5
17. Magalon J, Bausset O, Serratrice N, et al. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. *Arthroscopy*. 2014;30(5):629–638. doi:10.1016/j.arthro.2014.02.020
18. 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. doi:10.1177/0363546510387517
19. Oudelaar BW, Peerbooms JC, Huis In 't Veld R, Vochteloo AJH. Concentrations of blood components in commercial platelet-rich plasma separation systems: A review of the literature. *Am J Sports Med*. 2019;47(2):479–487. doi:10.1177/0363546517746112
20. Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol*. 2009;27(3):158–167. doi:10.1016/j.tibtech.2008.11.009
21. Delong JM, Russell RP, Mazzocca AD. Platelet-rich plasma: The PAW classification system. *Arthroscopy*. 2012;28(7):998–1009. doi:10.1016/j.arthro.2012.04.148
22. Zimmermann R, Arnold D, Strasser E, et al. Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. *Vox Sang*. 2003;85(4):283–289. doi:10.1111/j.0042-9007.2003.00361.x
23. Oh JH, Kim WOO, Park KU, Roh YH. Comparison of the cellular composition and cytokine-release kinetics of various platelet-rich plasma preparations. *Am J Sports Med*. 2015;43(12):3062–3070. doi:10.1177/0363546515608481
24. Mazzocca AD, McCarthy MB, Chowaniec DM, et al. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg Am*. 2012;94(4):308–316. doi:10.2106/JBJS.K.00430
25. Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res*. 2006;17(2):212–219. doi:10.1111/j.1600-0501.2005.01203.x
26. Marx RE. Platelet-rich plasma (PRP): What is PRP and what is not PRP? *Implant Dent*. 2001;10(4):225–228. doi:10.1097/00008505-200110000-00002
27. Mazzucco L, Balbo V, Cattana E, Guaschino R, Borzini P. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet®, RegenPRP-Kit®, Plateltex® and one manual procedure. *Vox Sang*. 2009;97(2):110–118. doi:10.1111/j.1423-0410.2009.01188.x
28. Królikowska A, Reichert P, Czamara A, Krzemińska K. Peak torque angle of anterior cruciate ligament-reconstructed knee flexor muscles in patients with semitendinosus and gracilis autograft is shifted towards extension regardless of the postoperative duration of supervised physiotherapy. *PLoS One*. 2019;14(2):e0211825. doi: 10.1371/journal.pone.0211825
29. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med*. 2011;39(10):2135–2140. doi:10.1177/0363546511417792
30. Cieślik-Bielecka A, Reichert P, Skowroński R, Królikowska A, Bielecki T. A new aspect of in vitro antimicrobial leukocyte- and platelet-rich plasma activity based on flow cytometry assessment. *Platelets*. 2019;30(6):728–736. doi:10.1080/09537104.2018.1513472
31. Kobayashi Y, Saita Y, Nishio H, et al. Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations. *J Orthop Sci*. 2016;21(5):683–689. doi:10.1016/j.jos.2016.07.009
32. 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(1). doi:10.2106/JBJS.L.00019
33. Zhang L, Chen S, Chang P, et al. Harmful effects of leukocyte-rich platelet-rich plasma on rabbit tendon stem cells in vitro. *Am J Sports Med*. 2016;44(8):1941–1951. doi:10.1177/0363546516644718

IV. PRACA NR 2:

Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods

Submit a Manuscript: https://www.f6publishing.com

World J Orthop 2022 June 18; 13(6): 587-602

DOI: 10.5312/wjo.v13.i6.587

ISSN 2218-5836 (online)

ORIGINAL ARTICLE

Basic Study

Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska

Specialty type: Orthopedics

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Haque N, Bangladesh; Yunus MHM, Malaysia

Received: January 18, 2022
Peer-review started: January 18, 2022
First decision: March 24, 2022
Revised: April 4, 2022
Accepted: May 7, 2022
Article in press: May 7, 2022
Published online: June 18, 2022

Maciej Dejnek, Jarosław Witkowski, Paweł Reichert, Department of Trauma Surgery, Wroclaw Medical University, Wroclaw 50-556, Poland

Helena Moreira, Department of Medical Science Foundation, Wroclaw Medical University, Wroclaw 50-556, Poland

Sylwia Płaczkowska, Teaching and Research Diagnostic Laboratory, Department of Laboratory Diagnostics, Wroclaw Medical University, Wroclaw 50-556, Poland

Piotr Morasiewicz, Institute of Medical Sciences, Department of Orthopaedic and Trauma Surgery, University of Opole, Opole 45-052, Poland

Aleksandra Królikowska, Ergonomics and Biomedical Monitoring Laboratory, Department of Physiotherapy, Wroclaw Medical University, Wroclaw 50-355, Poland

Corresponding author: Maciej Dejnek, MD, Research Scientist, Surgeon, Department of Trauma Surgery, Wroclaw Medical University, ul. Borowska 213, Wroclaw 50-556, Poland. maciej.dejnek@student.umed.wroc.pl

Abstract

BACKGROUND

The evaluation of the efficacy of platelet-rich plasma (PRP) in clinical practice yields conflicting results and raises numerous controversies. This may be due to different concentrations of biologically active components in PRP obtained with the use of different methods of gravity separation.

AIM

To compare the content, repeatability and correlations between biologically active components in PRP obtained with four different commercial systems.

METHODS

From a whole blood sample of each of 12 healthy male volunteers, 4 PRP samples were prepared using 4 different commercial kits [Arthrex Autologous Conditioned Plasma (ACP), Mini GPS III, Xerthra, Dr. PRP] in accordance with the instructions provided by the manufacturers. A comparative analysis of blood cell

components - 13 selected inflammatory cytokines and 7 growth factors - in the obtained PRP samples was performed using the Kruskal-Wallis test by ranks. The repeatability of results in each method was evaluated by the estimation of the coefficient of variation. The Spearman correlation was used to estimate the relationship between blood cell content and cytokines.

RESULTS

Significantly higher concentrations of platelets (PLT), white blood cells (WBC) and red blood cells (RBC) were found in PRP obtained with the use of Mini GPS III than in PRP obtained using other systems. Significant differences in the content of growth factors and cytokines in PRP were found. A positive correlation of the amount of PLT, RBC and WBC with the concentration of most of the growth factors was found but in only three inflammatory cytokines. The obtained correlations between blood cell components and cytokines differed between the systems in terms of statistical significance, which may be due to insufficient sample size. The repeatability of the obtained PLT concentration also varied between protocols with the lowest in Xerthra and the highest in Arthrex ACP.

CONCLUSION

Significant differences in the content of biologically active components and their repeatability were found in PRP obtained by various methods, providing new data for further research.

Key Words: Platelet-rich plasma; Cytokines; Chemokines; Growth factors

©The Author(s) 2022. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: The presented study showed important differences between blood cell components and levels of selected growth factors and inflammatory cytokines in platelet-rich plasma obtained with four different commercial preparation systems in a single-donor model. The range of cytokines analyzed far exceeded the ranges investigated in earlier publications. This was also the first study to pay attention to the repeatability of the quality of the obtained platelet-rich plasma (PRP). New positive correlations were found between platelet content in PRP and several cytokines (Hepatocyte growth factor, Interleukin-1β, Monocyte chemoattractant protein-1, Interleukin-8, Interleukin-18). The demonstrated positive correlation between red blood cell content in PRP and cytokines has never been described before.

Citation: Dejnek M, Witkowski J, Moreira H, Płaczkowska S, Morasiewicz P, Reichert P, Królikowska A. Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods. *World J Orthop* 2022; 13(6): 587-602

URL: https://www.wjgnet.com/2218-5836/full/v13/i6/587.htm

DOI: https://dx.doi.org/10.5312/wjo.v13.i6.587

INTRODUCTION

Autologous platelet-rich plasma (PRP) therapy is a method known for many years used in the treatment of various diseases. Nowadays, despite numerous controversies, it is widely used, especially in aesthetic medicine, orthopedics and sports medicine[1-6]. The method involves preparation from the patient's blood sample of plasma with a concentration of platelets higher than physiological and then its injection into pathological tissues[2,6-8].

In the human body, platelets are involved in the repairing processes, inter alia, by releasing different cytokines including growth factors from their α granules[2,6,9-11]. Inflammatory cytokines have also an important role in the initiation of the healing process, chemotaxis and keratinocyte proliferation but their excess can impair regeneration[12].

The essence of treatment with PRP is to accelerate the repairing processes by delivering a high concentration of platelet-derived growth factors and other cytokines directly to the affected area[6,13]. Attempts have been made to use PRP for treating diseases in which the repairing processes are impaired or when PRP was expected to accelerate healing as much as possible. This applies especially to chronic overuse injuries such as enthesopathies or tendinopathies, to the acceleration of the healing process of many different sports injuries of ligaments, tendons and muscles but also to bone union disorders and hard-healing wounds[6,13-16]. Intraarticular PRP injections used in osteoarthritis may be beneficial in the alleviation of chronic pain helping to increase the physical activity of patients[4,17-19].

The production of autologous platelet-rich plasma using commercial kits is a fast, convenient, affordable and safe way to obtain high concentrations of the desired growth factors[13,20]. By now it is known that too low platelet concentration is insufficient to induce a tissue response but too high a concentration can even have some negative impact on tissue healing[21,22].

There is an increasing number of manufacturers providing commercial kits that enable the quick, easy and safe preparation of PRP in an outpatient setting. Such kits differ from one another by some parameters such as the amount of material collected from the patient, the type of anticoagulant, the structure of the separator, the time and speed of centrifugation, the method of extraction and activation of the obtained plasma. They also differ in the assumed concentration that they allow to obtain and the presence of leukocytes in the final product[2,20,23-25]. Such a multitude of variables makes it impossible to reliably assess the effectiveness of therapies with different autologous platelet-rich plasma preparations without providing detailed information[21]. Furthermore, only a small number of studies on relatively small populations have shown significant differences in the desired cytokine content by testing only a few from many commercially available PRP systems. Despite many years of clinical use of PRP, there is still a remarkable scarcity of relevant and reliable information about its biologically active compounds. This lack of information was the main reason for the previous preliminary study conducted by the present authors[26]. Its results encouraged the authors to expand the study with a larger population and a greater amount of analyzed cytokines.

MATERIALS AND METHODS

Study aim, design and setting of the study

The goal of this study was to compare the content of blood cell components, selected inflammatory cytokines and selected growth factors in PRP samples obtained with the use of various commercial kits, to find any correlations between those biologically active components and to establish their repeatability within each method.

The study was designed as a single-center prospective non-randomized case-series descriptive laboratory study and was conducted in a medical university laboratory in the autumn of 2020 by clinicians from the Department of Trauma and Hand Surgery and the Department of Sports Medicine in cooperation with laboratory researchers from the Faculty of Pharmacy.

It was conducted in accordance with the standards of Good Clinical Practice. The study was approved by the institutional bioethics committee. All participants signed informed consent to participate in the study. The study followed the Minimum Information for Studies Evaluating Biologics in Orthopedics (MIBO) guidelines[27].

Characteristics of participants

The study involved 12 healthy male volunteers, aged 24-35 years (mean ± SD, 28.92 ± 3.15 y.o.). The mean height, body weight and body mass index were 182.42 ± 5.14 cm, 86.92 ± 8.73 kg and 26.16 ± 2.83 kg/m^2, respectively. The inclusion criteria were: the age of 20-35 years, the absence of serious diseases and conditions that could affect the amount and function of blood cell components (in particular diabetes, blood dyscrasia, inflammatory conditions, cancer, pre-existing joint pathologies). Volunteers who, in the two weeks preceding the study, were taking drugs that may interfere with the function of platelets or may affect the quantity or quality of blood cell components (in particular antiplatelet or anti-inflammatory drugs) were excluded from the study.

Each volunteer, after signing the informed consent, was asked to complete a questionnaire with personal information about age, height, weight, diseases, medications, sports activities and nicotine addiction. A vast majority of the respondents (91.67%) declared practicing sports at least once a week, and none of them regularly smoked cigarettes.

PRP preparation

Four commercially available systems for the preparation of PRP intended for use in orthopedics and sports medicine were selected: the Arthrex Autologous Conditioned Plasma (ACP) Double Syringe System (Arthrex Inc., United States), the Mini GPS III Platelet Concentration System (Biomet Inc., United States), the Xerthra PRP kit (Biovico Sp. z o.o., Poland), Dr. PRP (Rmedica, Republic of Korea).

Following the principles of asepsis, 74 mL of whole blood was collected and divided immediately into:

- 2 mL to a probe with ethylenediaminetetraacetic acid (EDTA).
- 13.5 mL to a double-syringe with 1.5 mL of anticoagulant citrate dextrose solution A (ACD-A) from Arthrex ACP.
- 27 mL to a syringe with 3 mL of ACD-A for the Mini GPS III kit.
- 13.5 mL to a syringe with 1.5 mL of 3.13% sodium citrate for the Xerthra kit.
- 18 mL to a syringe with 2 mL of 3.13% sodium citrate for the Dr. PRP kit.

The four samples of liquid-form PRP were prepared simultaneously from blood obtained from each volunteer using 4 different commercial kits according to the manuals provided by the manufacturers. The main characteristics of the PRP protocols are listed in Table 1 and illustrated in Figure 1.

Analysis of blood cell composition

First, the whole blood samples collected for EDTA were analyzed. The time between blood draw, PRP processing, extraction and activation did not exceed 1 h. All preparations were conducted in daylight and at room temperature. The whole blood count and blood cell composition of PRP samples were analyzed using an automatic laboratory analyzer Mindray BC-5150 (Shenzhen Mindray Bio-Medical Electronics Co., PRC) which needs 20 μL for each analysis. Immediately after PRP preparation, each sample was transferred into Eppendorf polypropylene tubes and then shaken gently for 30 s directly before analysis. Platelet capture efficiency (PCE) was calculated with the formula below, described previously by J. Magalon[23].

PCE (%) = [Volume of PRP obtained (mL) × platelet concentration in PRP (G/L)]/[Net volume of whole blood collected (mL) × platelet concentration in whole blood (G/L)].

Platelet activation and sample storage

The remaining PRP (1 ml) was dispensed into Eppendorf polypropylene tubes and then activated through a double freeze-thaw process (30 min for each step) according to the procedure described by R. Zimmermann[25]. The activated samples were frozen to the temperature of -80 °C and stored for further analysis.

Analysis of the content of inflammatory cytokines and growth factors

The samples were thawed to room temperature and centrifuged for 5 min at 2500 revolutions per minute in a Micro Star 17 microcentrifuge (VWR International Company, Thermo Electron LED, Germany) immediately before performing the composition analysis of selected cytokines using flow cytometry.

A LEGENDplex™ Custom Human 7-plex Panel (BioLegend, United States) was used to quantify the following platelet growth factors:

- Transforming Growth Factor-β1 (TGF-β1, free active).
- Epidermal growth factor (EGF).
- Fibroblast Growth Factor- basic (FGF-basic).
- Vascular endothelial growth factor (VEGF).
- Hepatocyte growth factor (HGF).
- Platelet-Derived Growth Factor-AA (PDGF-AA).
- Platelet-Derived Growth Factor-BB (PDGF-BB).

LEGENDplex™ Human Inflammation Panel 1 (BioLegend, United States) was used to quantitatively measure 13 human inflammatory cytokines:

- Interleukin-1β (IL-1β).
- Interferon-α2 (IFN-α2).
- Interferon-γ (IFN-γ).
- Tumor Necrosis Factor α (TNF-α).
- Monocyte Chemoattractant Protein-1 (MCP-1; CCL2).
- Interleukin-6 (IL-6).
- Interleukin-8 (CXCL8).
- Interleukin-10 (IL-10).
- Interleukin-12p70 (IL-12p70).
- Interleukin-17A (IL-17A).
- Interleukin-18 (IL-18).
- Interleukin-23 (IL-23).
- Interleukin-33 (IL-33).

BioLegend's LEGENDplex™assays are bead-based multiplex immunoassays that use fluorescence-encoded beads and flow cytometer measurements. The concentrations of particular cytokines were determined by means of a standard curve generated during the performance of the test. The analyses were conducted according to the manufacturer's procedure. The samples were acquired on CyFlow SPACE and CyFlow CUBE flow cytometer (Sysmex-Partec, Germany) using a 488 nm laser with a 536/40 (BP) filter for the PE fluorochrome, and a 638 nm laser with 675/20 (BP) for the APC fluorochrome. The results were analyzed with LEGENDplex™ Data Analysis Software V.8.0 (Vigene Tech Inc., United States).

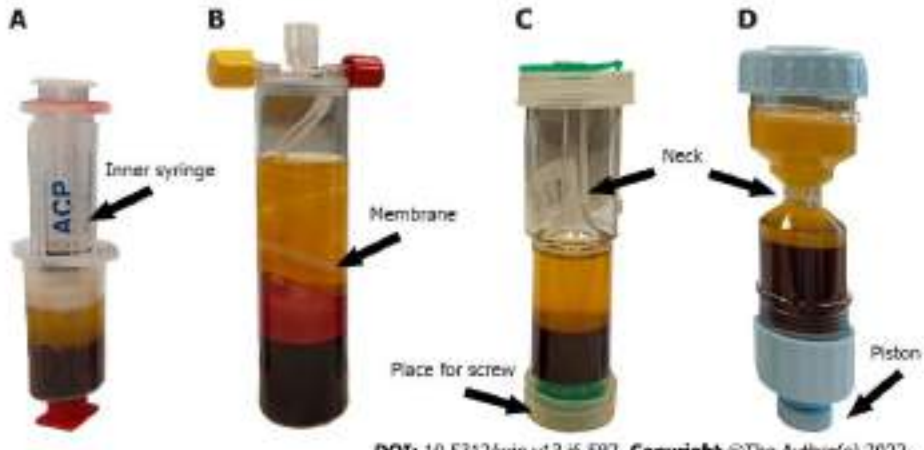
Statistical analysis

The results were statistically analyzed using Statistica 13.3 software (TIBCO Software Inc, United States). Arithmetic means ± SD were calculated. The Shapiro-Wilk test was used to analyze the compliance of the distribution of the analyzed variables with normal distribution. The Kruskal-Wallis test by ranks (one-way analysis of variance on ranks) with Dunn's post-hoc test was used to compare

Table 1 The main characteristics of the platelet-rich plasma protocols used in the study

	Arthrex ACP	Mini GPS III	Xerthra	Dr. PRP
Amount of blood collected	13.5 mL	27 mL	13.5 mL	18 mL
Final amount of PRP	4 mL	3 mL	1.5 mL	3 mL
Drawn blood/obtained PRP	3.38:1	9:1	9:1	6:1
Anticoagulant type	ACD-A	ACD-A	3.13% SOD CITR	3.13% SOD CITR
Anticoagulant amount	1.5 mL	3 mL	1.5 mL	2 mL
Number of spins	1	1	1	1
RPM	1500	3200	3500	3100
Centrifugation time	5'	15'	5'	4'
Removal of PPP	no	yes	yes	yes
Expected WBC content	low	high, 5x	low	low
Expected PLT concentration	2-3 x	9.3 x	2-13 x	4-5 x
Dedicated centrifuge	Yes	Yes	Yes	Yes
Activation method	*In vivo*	*In vivo*	*In vivo*	*In vivo*
Type of PRP	Conditioned Plasma	LR-PRP	LP-PRP	LP-PRP

ACD-A: Anticoagulant citrate dextrose solution A; LR-PRP: Leukocyte-rich platelet-rich plasma; LP-PRP: Leukocyte-poor platelet-rich plasma; PPP: Platelet-poor plasma; RPM: Revolutions per minute; SOD CITR: Sodium citrate.



DOI: 10.5312/wjo.v13.i6.587 Copyright ©The Author(s) 2022.

Figure 1 Different platelet-rich plasma kits after centrifugation. Highlight specific items in each kit used for platelet-rich plasma (PRP) extraction. A: Arthrex autologous conditioned plasma, the prepared plasma is transferred by pulling it into the inner syringe in a double-syringe system; B: Mini GPS III, PRP is automatically separated during centrifugation by a special membrane in the separator; C: Xerthra, the controller screw placed at the bottom is used to move the PRP to the neck of the tube; D: Dr. PRP, the piston is used to push PRP to the tube's neck.

the concentrations of platelets (PLT), white blood cells (WBC), red blood cells (RBC) and cytokines between each protocol. Spearman's rank correlation coefficient was used to find any relationship between blood cell components and cytokines. The strength of the correlation was determined for ρ-value: negligible for $\rho \leq 0.2$, week for $\rho \leq 0.4$, moderate for $\rho \leq 0.6$, strong for $\rho \leq 0.8$ and very strong for $\rho > 0.8$. The repeatability of the results obtained for each of the methods was estimated as the coefficient of variation (CV) which is the ratio of the standard deviation to the mean, multiplied by 100%. High repeatability was established as a CV < 25%, moderate as a CV = 25%-44%, weak as a CV = 45%-99% and very weak as a CV > 100%. Statistical significance was established at the level of $P \leq 0.05$.

Power analysis for PRP comparison was performed using automatic software calculation for several means one-way ANOVA with the use of Root Mean Square Standardized Effect. The statistical power of the tests was set at 0.8. Based on the magnitude of the effect calculated according to the preliminary study, it was estimated that in order to show significant blood cell component differences between the PRP groups with a test power of 0.8, each group should have a minimum of 6 subjects. Similarly, the

population needed to show differences in GF levels differs depending on the factor tested (from 8 to 61 subjects). Group size could not be estimated for differences in inflammatory cytokine levels as there were no previous studies to provide the necessary data. The authors calculated that 48 PRP samples divided into four groups should be sufficient to achieve the assumed goal of the study.

RESULTS

Whole blood count

The blood counts of all participants are shown in the Table 2.

Blood cell components of PRP samples

The highest concentrations of PLT, WBC and RBC in PRP were obtained with Mini GPS III. Platelet concentration in PRP obtained with Mini GPS III was significantly higher than that obtained with Artherx ACP ($P < 0.001$), Xerthra ($P < 0.001$) and Dr. PRP ($P < 0.008$). The differences between the remaining systems were not significant ($P > 0.05$). The situation was similar with the ability to concentrate PLT above the baseline with 5.05 × for Mini GPS III which was significantly higher than for other systems, for which it ranged from 1.47 to 2.14 ($P < 0.05$). Four PRP samples prepared on Xerthra did not exceed the whole blood baseline level of PLT concentration and the other four exceeded it by more than 2 times. Only one sample prepared with Dr. PRP and none of the samples prepared with Mini GPS III and Arthrex failed to exceed the baseline level.

WBC concentration and neutrophil count also significantly differ only when comparing Mini GPS III with other systems ($P < 0.005$) but they do not differ significantly between those other systems ($P > 0.05$). Lymphocytes, monocytes, eosinophils and basophils were on a detectable level only in Mini GPS III PRPs.

The highest RBC contamination in the samples was observed for Mini GPS III and it was significantly higher compared to other systems ($P < 0.001$). RBC concentrations in Arthrex ACP, Xerthra and Dr. PRP were all barely detectable and amounted to 0.05, 0.02 and 0.01 × 10^{12}/L, respectively.

Several means one-way ANOVA power analysis of these multiple comparisons reached levels above 0.99. All blood cell components are shown in Table 3.

Platelet capture efficiency

PCE values, in descending order, were obtained for Mini GPS III at 56.15% ± 7.44%, Arthrex ACP at 43.68% ± 5.32%, Dr. PRP at 35.61% ± 12.13% and for Xerthra at 21.79% ± 18.98% (Figure 2). Statistical analysis showed significant differences only between Mini GPS III and Xerthra ($P < 0.001$) and Dr. PRP ($P = 0.001$).

Repeatability of the obtained concentrations in PRP samples

The coefficient of variation (CV) showed the highest repeatability of PLT concentrations for Arthrex ACP (12.18%) and the Biomet GPS III system (13.25%). The least predictable PLT concentrations were provided by the Xerthra PRP kit (95.95%). The results of CV for WBC and RBC concentrations seem noteworthy only for LR-PRP obtained for Mini GPS III. The repeatability was moderate for WBC (CV = 26.79%) and weak for RBC (CV = 56%). All CV results are shown in Table 4.

The concentrations of growth factors and inflammatory cytokines in PRP samples

The highest concentrations of EGF, VEGF, HGF, PDGF-AA and PDGF-BB were found in PRP samples obtained with Mini GPS III and the lowest in samples obtained with Arthrex ACP, and the differences for the first four were statistically significant with P values = 0.005, 0.02, 0.01 and 0.006, respectively. A statistically significant difference was also found between Mini GPS III and Xerthra in the concentration of EGF ($P = 0.04$) and PDGF-AA ($P = 0.04$). Several means one-way ANOVA power analysis of these multiple comparisons showed insufficient levels (< 0.8) for TGF-β1, FGF-basic, VEGF, HGF and PDGF-BB suggesting the insufficient sample size in this regard. Mean results with standard deviations of all tested growth factors are included in Supplementary Table 1 and highlighted in Figure 3.

Among all tested inflammatory cytokines, statistically significant differences between systems were found only in the levels of IL-8 and IL-18. IL-8 concentration in PRP obtained with Mini GPS III (734.85 pg/mL) was higher than in that obtained with Arthrex ACP (139.53 pg/mL, $P = 0.02$) and the Xerthra PRP kit (122.98 pg/mL, $P = 0.004$). IL18 concentration was the highest in PRP from MiniGPS III (1377 pg/mL) with a significant difference compared to Arthrex ACP (509.41 pg/mL, $P = 0.04$), the Xerthra PRP kit (283.01 pg/mL, $P < 0.001$) and Dr. PRP (414.02 pg/mL, $P = 0.007$). Unfortunately, several means one-way ANOVA power analysis of these multiple comparisons showed levels above 0.8 only for IL-18, which makes correct interpretation of the obtained results much more difficult. Mean results with standard deviations of all the tested cytokines are included in Supplementary Table 1 and highlighted in Figure 4.

Table 2 Whole blood count with differential leukocyte of all participants

Differential leukocyte	Blood count
RBC (10^{12}/L)	4.97 ± 0.43
PLT (10^9/L)	240.67 ± 49.85
WBC (10^9/L)	6.49 ± 1.49
Neutrophils (10^9/L)	3.79 ± 1.29
Lymphocytes (10^9/L)	2.08 ± 0.45
Monocytes (10^9/L)	0.45 ± 0.13
Eosinophils (10^9/L)	0.14 ± 0.08
Basophils (10^9/L)	0.04 ± 0.01

RBC: Red blood cells; PLT: Platelets; WBC: White blood cells.

Table 3 Concentrations of blood cell components in platelet-rich plasma samples

	Arthrex ACP	Mini GPS III	Xerthra	Dr. PRP
PLT (10^9/L)	357.33 ± 99.01	1212.67 ± 268.63	455.27 ± 362.92	499.75 ± 153.46
WBC (10^9/L)	0.87 ± 1.01	34.19 ± 11.18	1.80 ± 2.55	0.60 ± 0.87
Neutrophils (10^9/L)	0.87 ± 1.01	16.71 ± 9.89	1.80 ± 2.55	0.60 ± 0.87
RBC (10^{12}/L)	0.05 ± 0.08	1.49 ± 0.86	0.02 ± 0.02	0.01 ± 0.01
xPLT	1.47 ± 0.18	5.05 ± 0.67	1.96 ± 1.71	2.14 ± 0.73
xWBC	0.14 ± 0.17	5.27 ± 1.41	0.29 ± 0.4	0.10 ± 0.16
xRBC	0.01 ± 0.02	0.30 ± 0.17	0.00	0.00

PLT: Platelets; WBC: White blood cells; RBC: Red blood cells; ACP: Autologous Conditioned Plasma; PRP: Platelet-rich plasma.

Table 4 The coefficient of variation in the concentration of blood cell components for different platelet-rich plasma preparation systems

	WBC	RBC	PLT
Arthrex ACP [%]	114.80	175.69	12.18
Mini GPS III [%]	26.79	56.83	13.25
Xerthra [%]	149.38	133.98	95.95
Dr. PRP [%]	151.45	95.10	34.05

ACP: Autologous Conditioned Plasma; WBC: White blood cells; RBC: Red blood cells; PLT: Platelets; PRP: Platelet-rich plasma.

Correlation between blood cell components and cytokines

Significant positive correlations of PLT, WBC and RBC concentrations with the following growth factors: EGF, VEGF, HGF, PDGF-AA, PDGF-BB were found. Most of them were weak or moderate. A strong Spearman correlation was found between PLT and EGF ($\rho = 0.602$, $P < 0.001$), PLT and PDGF-AA ($\rho = 0.637$, $P < 0.001$). All correlations are presented in Supplementary Figure 1.

Positive significant correlations of PLT, WBC and RBC concentrations with the following inflammatory cytokines: IL-1β, MCP-1, IL-8, IL-18 were found. A strong Spearman correlation was found only between PLT and IL-18 ($\rho = 0.627$, $P < 0.001$). All correlations are presented in Supplementary Figure 1.

The correlations between blood cell components and growth factors or cytokines in PRP samples vary widely between different systems. In PRP samples obtained with Arthrex ACP, a significant Spearman correlation was found between PLT and PDGF-AA, INF- γ, IL-8. In PRP samples prepared with Mini GPS III System, a significant positive correlation was found between RBC and IL-1β, IL-18. In PRP prepared with Xerthra, a significant positive correlation was found between PLT and EGF, VEGF, PDGF-AA, IL-18; between WBC and TGF-β1, EGF, VEGF, PDGF-AA; between RBC and EGF, VEGF,

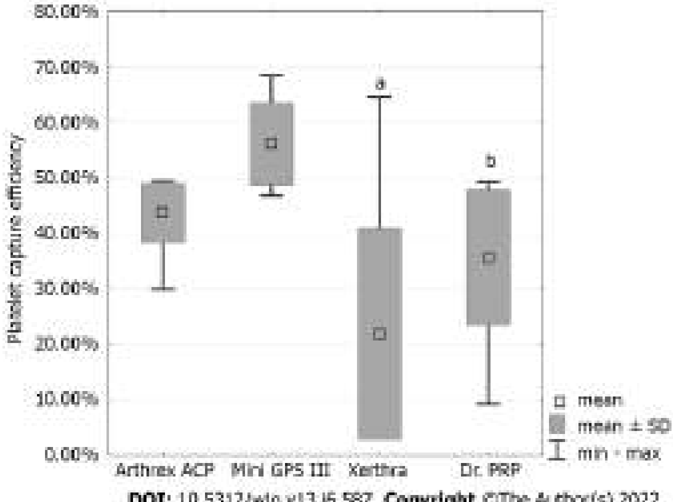


Figure 2 Platelet capture efficiency (%) of different preparation protocols. $^{a}P < 0.001$ *vs* Mini GPS III; $^{b}P = 0.001$ *vs* Mini GPS III. Arthrex ACP: Arthrex Autologous Conditioned Plasma.

PDGF-AA. In PRP samples prepared with Dr. PRP Kit, a significant correlation was found between PLT and EGF, FGF-basic; between WBC and IL-18, IL-23. All significant Spearman correlation values are presented in Supplementary Table 2. Selected differences in correlations obtained with different systems are shown in Figure 5.

DISCUSSION

The results of the study revealed significant differences in PRP obtained with various commercial kits. Other studies had already confirmed the existence of such differences, therefore it was not the essence of the study[20]. The main goal was to highlight these differences, especially in the case of kits that have not been tested in this way before, such as Xerthra and Dr. PRP. Another novelty was the range of the investigated cytokines, significantly exceeding the ranges investigated in previous publications[10,20, 23]. This study was also the first to pay attention to the repeatability of the quality of the obtained PRP, which is particularly important when planning a therapy or a clinical trial. This study also showed interesting differences in the results of PRP analysis and the correlations found as compared to the available literature.

It is believed that for therapeutic effect, PRP should have a platelet concentration above the baseline level. All PRP preparation systems were able to produce PRP with the mean platelet concentrations above this level but 4 samples prepared using Xerthra and 1 sample prepared using Dr. PRP did not meet this criterion. It seems to be due to the construction of separators in these systems. Immediately after centrifugation, the PRP is not physically separated from PPP and RBC which is why during the separation and extraction process it is not difficult to partially mix the content. The Arthrex ACP system uses a different approach because after centrifugation it has only two layers - RBC and easily removable plasma. That is probably why the manufacturer called its product Autologous Conditioned Plasma instead of Platelet-Rich Plasma. In his paper, L. Mazzucco considered PLT > 200 × 10^3/mL as sufficient for therapeutic effect, and all investigated systems reached this value[28]. The US Food and Drug Administration (FDA) requires a platelet concentration of at least 250 × 10^3/mL for PRP products which was also achieved by all systems. Other authors recommend a PLT concentration of about 1000 × 10^3[29]. Among systems included in this study, only Mini GPS III meets this criterion.

The content of PLT in PRP differed among systems but this difference was significant only when comparing Mini GPS III to other systems. The results of this study concerning PLT concentration for Mini GPS III and Arthrex ACP were similar to those obtained by other researchers[10,23,30,31]. The present authors found a positive correlation of PLT content with EGF, VEGF, HGF, PDGF-AA, PDGF-BB, IL-1β, MCP-1, IL-8 and IL-18 but not with FGF-basic, TGF-1β and the rest of the investigated inflammatory cytokines. Other authors support the correlation of PLT in PRP with PDGF[20], PDGF, VEGF [10], PDGF-AB, VEGF, EGF[23], PDGF-AB[31]. Contrary to the results presented in this study, J. Magalon and E.A Sundman found a positive correlation of PLT with TGF1β[23,31]. According to the present authors' knowledge, the positive correlations of PLT with MCP-1, IL-8 and IL-18 in PRP samples are presented in a scientific paper for the first time.

There are some controversies about leukocyte-rich platelet-rich plasma which is expected to have WBC concentration above the baseline. Its negative effect on tissue healing was demonstrated in *in vitro*

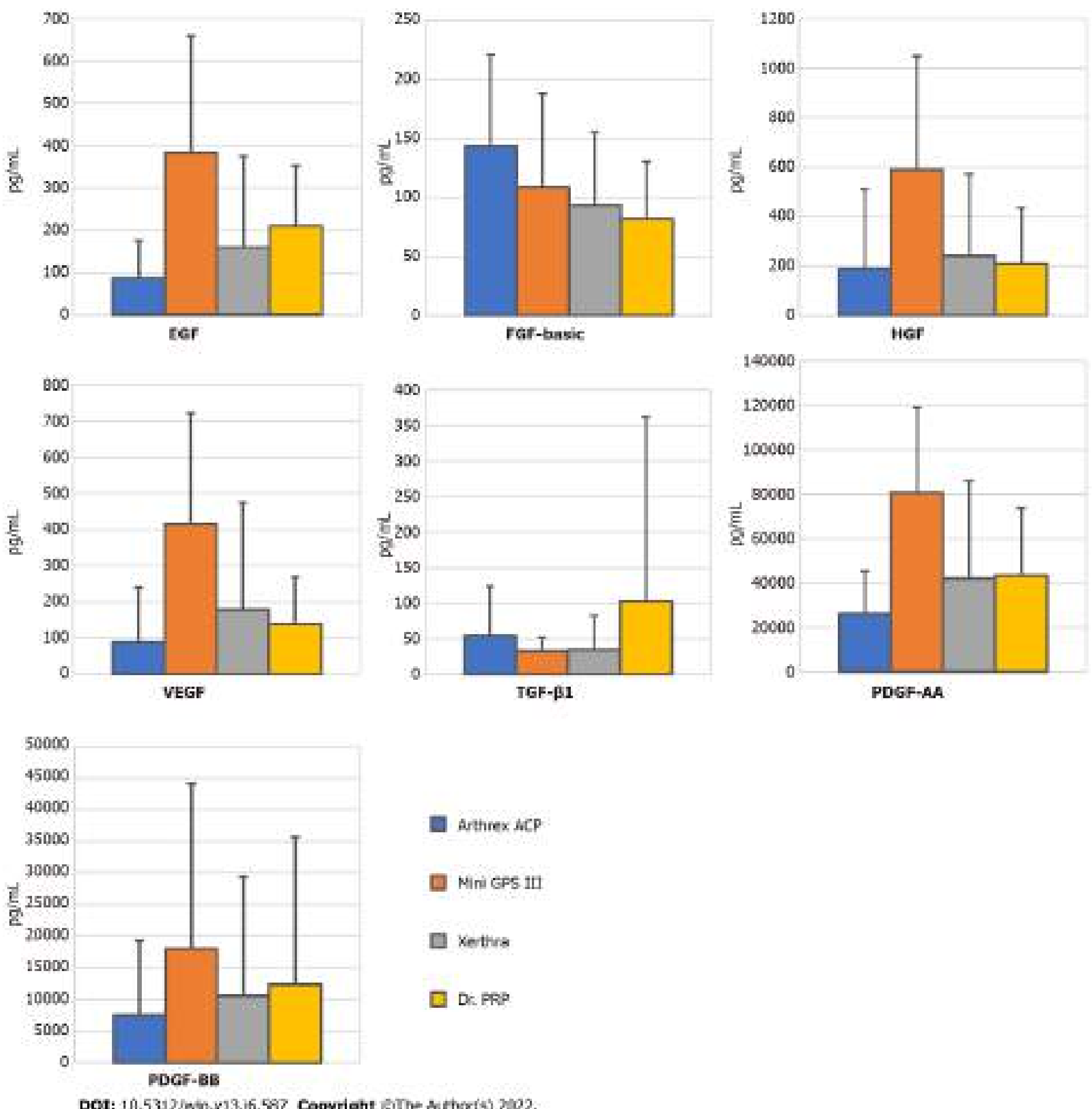
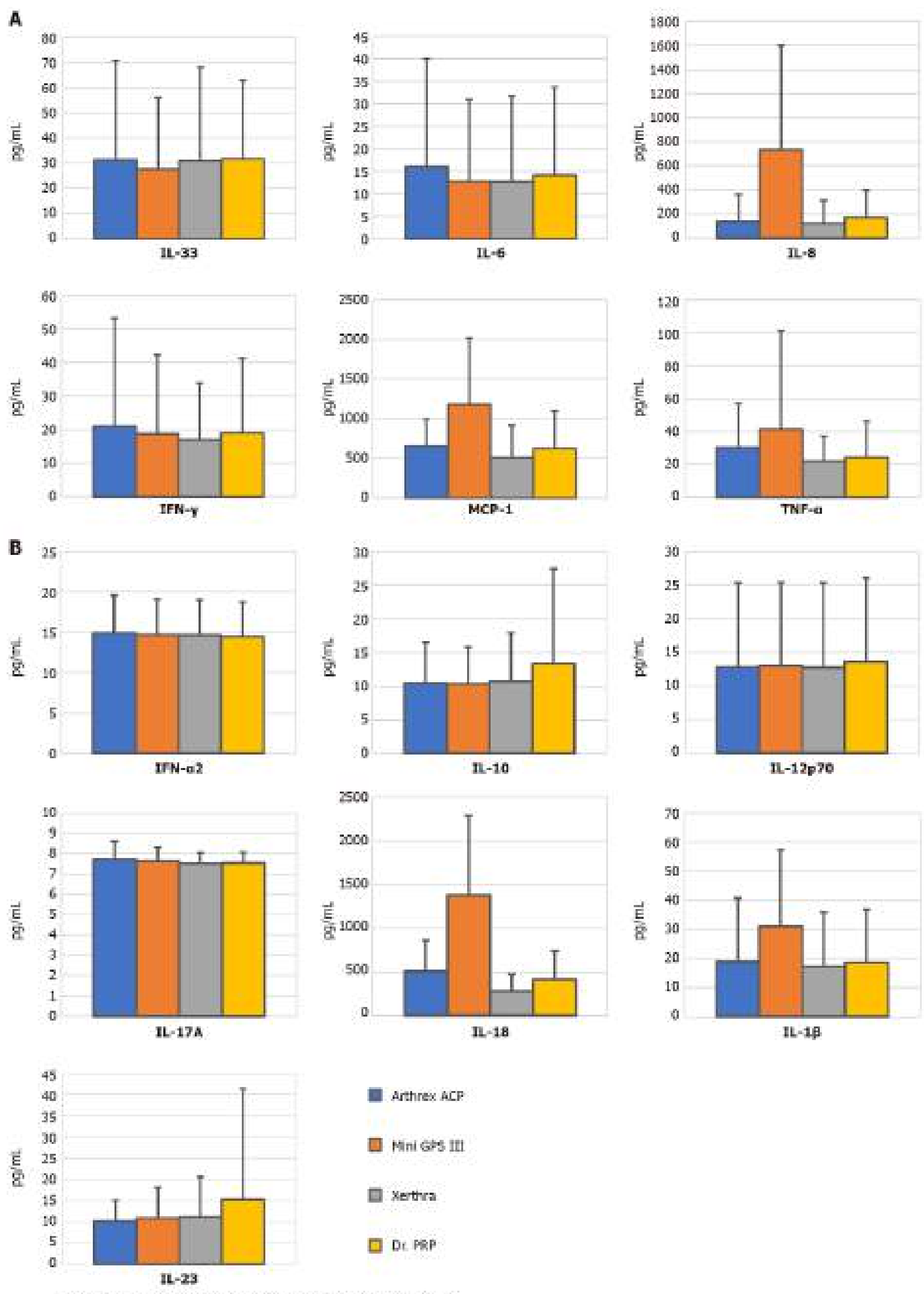


Figure 3 Concentrations (pg/mL) of growth factors in platelet-rich plasma samples obtained by different systems (mean ± SD). EGF: Epidermal growth factor; FGF-basic: Fibroblast growth factor-basic; HGF: Hepatocyte growth factor; VEGF: Vascular endothelial growth factor; TGF-β1: Transforming growth factor-β1; PDGF-AA: Platelet-derived growth factor-AA; PDGF-BB: Platelet-derived growth factor-BB.

studies, which, however, was not confirmed by studies on living organisms. This effect may be due to the high content of proteinases and hydrolases in neutrophils and should be taken into consideration when planning the therapy[8,13,31,32]. WBC content could also have an antibacterial effect[33]. On the other hand, in the presented study, high leukocyte content positively correlates with the levels of important growth factors and cytokines such as EGF, VEGF, HGF, PDGF-AA, PDGF-BB, IL-1β, MCP-1, IL-8 and IL-18. Authors of similar papers do not frequently report results of correlations between WBC and GF. J. Magalon has shown a significant positive correlation of WBC content with EGF and VEGF but not with PDGF-AB and TGF-β1[23]. T. N. Castillo also found a positive correlation of WBC with VEGF and PDGF-ββ but not with PDGF-AB or TGF-β1[24]. The presented results support and greatly extend the above. The highest WBC content was found in PRP prepared using the Mini GPS III System as it is defined as leukocyte-rich platelet-rich plasma (L-PRP)[21,34].

There was also significantly higher content of RBC in Mini GPS III than in other systems. This is undesirable evidence of imperfect separation of PRP and may explain why other authors did not provide detailed information about RBC and its correlation with growth factors and cytokines. Surprisingly, a positive correlation was again demonstrated with EGF, VEGF, HGF, PDGF-AA, PDGF-BB, IL-1β, MCP-1, IL-8 and IL-18. This has never been reported by other authors in the context of PRP



DOI: 10.5312/wjo.v13.i6.587 Copyright ©The Author(s) 2022.

Figure 4 Concentrations (pg/mL) of inflammatory cytokines in platelet-rich plasma samples obtained by different systems (mean ± SD). A: Mini GPS III; B: Arthrex Autologous Conditioned Plasma. IFN-α2: Interferon-α2; IL-10: Interleukin-10; IL-12p70: Interleukin-12p70; IL-17A: Interleukin-17A; IL18: Interleukin-18; IL-1β: Interleukin-1β; IL-23: Interleukin-23; IL-33: Interleukin-33; Il-6: Interleukin-6; IL-8: Interleukin-8; IFN-γ: Interferon-γ; MCP-1: Monocyte

chemoattractant protein-1; TNF-α: Tumor necrosis factor α.

evaluation and requires further research.

Significant differences in the levels of EGF, VEGF, HGF, PDGF-AA, PDGF-BB, IL-8 and IL-18 were found between Mini GPS III and Arthrex, for EGF, IL-8 and IL-18 between Mini GPS III and Xerthra and for IL-18 between Mini GPS III and Dr. PRP. The presented results support and extend the current state of knowledge[10,23,24,30,31,35]. Further studies are needed to explain the origin of these differences in the cases in which the correlation between blood cell components and cytokines/growth factors cannot simply explain it.

For the first time in the literature, the repeatability of obtained concentrations of PLT, WBC and RBC in different PRP preparation systems was clearly evaluated. The presented study demonstrated considerable differences between systems. In the authors' opinion, especially Xerthra PRP kit requires some improvements in the provided protocol for better repeatability. Due to the fact that two PRP samples prepared with Xerthra had a PLT increase > 4x above the baseline level, the authors still believe that there is great potential in this system.

For clinical practice and further studies, the most important issue is what should be expected from differences in PRP cytokine levels. Multiple studies have demonstrated a beneficial effect of PDGF and FGF on the healing process, both in animal models and in patients with wound healing disorders. However, *in vivo* functions of many growth factors remain largely unconfirmed[12]. In this study, PDGF-AA and PDGF-BB were selected as representatives of the PDGF family which stimulates cell (neutrophil, monocyte, fibroblast) migration to the wound site, enhances the proliferation of fibroblasts and production of extracellular matrix[6,12]. FGF-basic, as an FGF family member has well described mitogenic activity, regulates migration and cell differentiation, and has a cytoprotective effect on cells under stress conditions[12]. VEGF is involved in the regulation of angiogenesis during wound healing, HGF was discovered as a stimulator of dissociation of epithelial cells, migration, proliferation and new blood vessel formation, EGF induces cell differentiation of both ectodermal and mesodermal origin, and TGF-β1 has an important role in controlling cell proliferation and differentiation during the repairing process[6,12]. Other important cytokines that play a positive role in tissue healing are MCP-1 as a major macrophage chemoattractant, IL-8 as a neutrophil chemoattractant and stimulant of reepithelialization, IL-10 as an inhibitor of inflammation and scar formation[12]. Proinflammatory cytokines such as IL-1α and β, IL-6 and TNF-α are also involved in the repairing process by stimulation of keratinocyte and fibroblast proliferation, synthesis and breakdown of extracellular matrix proteins, fibroblast chemotaxis and regulation of the immune response[12].

It is expected that high levels of the above cytokines in PRP should result in better wound healing. In the presented study, a positive but not always significant Spearman correlation was found between all blood cell components and growth factors such as PDGF-AA, PDGF-BB, EGF, VEGF, HGF, MCP-1, IL-8 and IL-1 β, however, their correlation with TGF-β1, FGF-basic, TNF-α, IL-6 and IL-10 was found to be negligible. These results suggest that the best clinical outcome should be expected from PRP with the highest concentration of blood cell components.

Proinflammatory cytokines such as IFN-α2, IFN-γ, IL-12p70, IL-17A, IL-18, IL-23 and IL-33 may play an additional role in wound healing by regulating the secretion of other cytokines, regulating the immune response and antimicrobial activity. However, their function in tissue repair is poorly understood. In the presented study, a significant correlation was found only between all blood cell components and IL-18. Since IL-18 is able to induce severe inflammatory reactions, the implications of its correlation with highly concentrated PRP require further investigation.

Significant differences in the content of blood cell components and growth factors between different methods of platelet-rich plasma separation have already been demonstrated in previous publications[10,20,23,24,30,35]. However, none of the studies analyzed the Xerthra and Dr. PRP systems in such great detail. The medical market offers much more commercially available PRP preparation systems than those included in the study. The present authors evaluated only four of them but still, most of similar studies analyzed a smaller or comparable number of different systems[10,23,24,30,35].

Another limitation of the study is the size of the population. However, the number of participants is larger compared to the majority of similar studies[20,23,24,30,35]. The group was kept as homogeneous as possible to eliminate additional factors that could distort the results, therefore the study included a group of healthy men of similar age. The post-hoc test power analysis showed a sufficient sample size for the PRP group comparison of PCE, blood cell components, EGF, PDGF-AA, IL-18 and slightly insufficient for VEGF, HGF, MCP-1 and IL-8. To achieve the test power level of 0.8 in order to compare most other cytokines, the sample size should be considerably increased from 3 (FGF-basic) to more than 100 times (IFN-α2, IL-12p70 or IL-33). This data may help other researchers to design their studies appropriately.

In the study presented in this paper, the degree of platelet activation was not tested. This was based on the available literature which showed no significant differences in platelet activation between different separation methods[23,36]. Moreover, according to the literature, the storage time of PRP in which there is no significant activation of the platelets, should not exceed 6 h at a temperature of 20°C

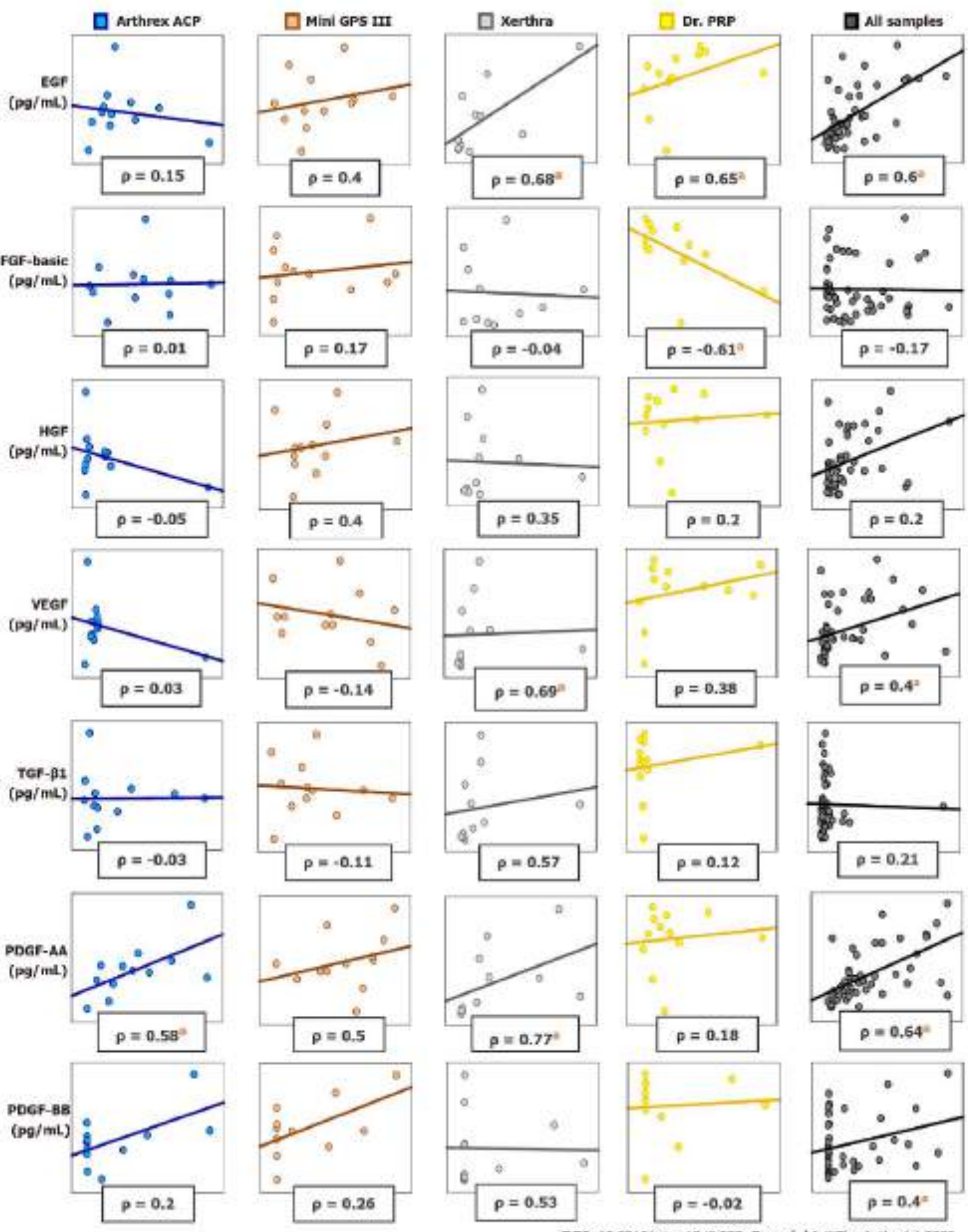


Figure 5 Spearman correlations (ρ) between platelets and growth factors in platelet-rich plasma obtained with different protocols and combined. aP < 0.05. EGF: Epidermal growth factor; FGF-basic: Fibroblast growth factor-basic; HGF: Hepatocyte growth factor; VEGF: Vascular endothelial growth factor; TGF-β1: Transforming growth factor-β1; PDGF-AA: Platelet-derived growth factor-AA; PDGF-BB: Platelet-derived growth factor-BB.

[37]. All PRP samples obtained in this study were tested for the content of blood cell components immediately after production and then they were frozen at -80 °C waiting for cytokine analysis.

Not all of the known cytokines and growth factors that may affect the healing and regeneration processes occurring in platelet-rich plasma were analyzed. However, the focus was on the most important ones. This study analyzes a wider range of cytokines than most publications on this topic[10,20,23,24,30,35].

CONCLUSION

Significant differences were found in the content of biologically active components in PRP obtained with the use of different methods and considerable differences were demonstrated in their repeatability within each method. Significant correlations between blood cell components and growth factors/inflammatory cytokines were presented, contributing new data to the current state of knowledge.

Clinical relevance and recommendations

Due to the considerable heterogeneity of PRPs, the presented results support the recommendations for studies reporting the use of autologous blood-based therapies to provide detailed information about the characteristics of all PRP samples (MIBO)[27]. The study also provides detailed information about the desired content of growth factors and their correlation with blood cell components in four different commercially available systems for PRP preparation, which could help clinicians to choose one depending on their expectations. The results of this study also demonstrate the need for producers to improve the existing solutions in order to improve the provided protocols to increase the repeatability of the parameters of PRP samples.

In clinical practice, it seems reasonable to suggest the use of commercial PRP kits that allow obtaining the highest blood cell concentration with the highest predictable reproducibility. It is worth noting that not only the level of PLT but also WBC and RBC levels significantly correlated with cytokines involved in the healing process, which may encourage researchers to look for new "blood cell concentrate" treatment methods.

ARTICLE HIGHLIGHTS

Research background

Autologous platelet-rich plasma (PRP) therapy is a method used to treat a variety of diseases related to soft tissue degeneration. The main idea behind this is to improve local healing and stimulate regeneration by administering large amounts of platelet-derived growth factors and cytokines. There are many commercial kits available to assist in obtaining PRP in an outpatient setting.

Research motivation

Due to the wide variety of PRP preparation systems, there are justified doubts about the quality of the obtained samples. Differences in the content of biologically active compounds between some PRP systems have already been demonstrated. However, only a small number of available systems and a limited number of cytokines and growth factors have been investigated.

Research objectives

To compare PRP obtained using four different commercial preparation systems in terms of the content of biologically active components, correlations between those components and their repeatability in each method.

Research methods

After obtaining informed consent from participants, whole blood was collected from 12 young healthy male volunteers, and 4 different PRP samples were prepared from each of them in a single-donor model. PRP samples were prepared using different commercial kits: Arthrex Autologous Conditioned Plasma (ACP) Double Syringe System (Arthrex Inc., United States), the Mini GPS III Platelet Concentration System (Biomet Inc., United States), the Xerthra PRP kit (Biovico Sp. z o.o., Poland) and Dr. PRP (Rmedica, Republic of Korea). The content of cellular components in each sample was assessed using an automatic laboratory analyzer Mindray BC-5150 (Shenzhen Mindray Bio-Medical Electronics Co., PRC). To quantify the content of seven selected growth factors (Epidermal growth factor (EGF), Fibroblast Growth Factor- basic, Hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), Transforming Growth Factor-β1, Platelet-Derived Growth Factor-AA, Platelet-Derived Growth Factor-BB and thirteen inflammatory cytokines [(Interferon-α2, Interleukin-10, Interleukin-12p70, Interleukin-17A, Interleukin-18 (IL-18), Interleukin-23 (IL-23), Interleukin-33, Interleukin-6, Interleukin-8, Interferon-γ, Monocyte Chemoattractant Protein-1 (MCP-1), Tumor Necrosis Factor α (TNF-α)], bead-based multiplex immunoassays LEGENDplexTM (BioLegend, United States) that use fluorescence-

encoded beads and flow cytometer measurements were performed.

Research results

Differences between PRPs obtained with various preparation systems were found in terms of cellular composition, repeatability, platelet capture efficiency, concentrations of growth factors and inflammatory cytokines. The highest ability to concentrate platelets (PLT) above the baseline was obtained with Mini GPS III (5.05 x) and the lowest with Arthrex ACP (1.47 x). Those two systems had the best repeatability of platelet concentrations assessed as the coefficient of variation of 13.25% and 12.18%, respectively. The highest concentrations of Epidermal growth factor, hepatocyte growth factor, vascular endothelial growth factor, platelet-derived growth factor-AA, platelet-derived growth factor-BB, IL-18, Interleukin-1β, Interleukin-8, MCP-1 and TNF-α were found in PRP with the highest PLT, white blood cells and red blood cells concentrations (obtained with Mini GPS III), and positive significant ($P < 0.05$) correlations between cell components and these paracrine factors (except TNF-α) were revealed.

Research conclusions

The study provided new data on the differences between PRP obtained with the various commercial systems. The range of analyzed cytokines far exceeded the ranges investigated in earlier publications. The presented findings should help researchers and clinicians choose the system that best meets their expectations.

Research perspectives

Further research should be focused on the comparison of PRPs obtained using different techniques in the context of their biological effect on soft tissues *in vitro* and their clinical efficacy in various diseases.

FOOTNOTES

Author contributions: Dejnek M, Reichert P, and Królikowska A designed and coordinated the study; Dejnek M, Witkowski J, Moreira H, and Placzkowska S performed the experiments, acquired and analyzed data; Dejnek M, Reichert P, Królikowska A, and Morasiewicz P interpreted the data; Dejnek M wrote the manuscript; all authors approved the final version of the article.

Supported by the Wroclaw Medical University as a Regional Center of Excellence in the field of medical sciences and health sciences implemented under the funds of the Ministry of Science and Higher Education (Republic of Poland) in the program "Regional Initiative of Excellence" in the years 2019-2022, No. RID.Z501.20.008.

Institutional review board statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of Wroclaw Medical University (KB - 163/2020, 30.03.2020).

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors declare no conflict of interest. The founders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data sharing statement: The datasets used during the current study are available from the corresponding author on reasonable request.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: Poland

ORCID number: Maciej Dejnek 0000-0002-6675-0256; Jaroslaw Witkowski 0000-0002-2754-1339; Helena Moreira 0000-0002-8084-3686; Sylwia Placzkowska 0000-0002-1466-3820; Piotr Morasiewicz 0000-0002-7587-666X; Pawel Reichert 0000-0002-0271-4950; Aleksandra Królikowska 0000-0002-6283-5500.

S-Editor: Wang LL
L-Editor: A

P-Editor: Li X

REFERENCES

1 **Creaney L**, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. *Br J Sports Med* 2008; **42**: 314-320 [PMID: 17984193 DOI: 10.1136/bjsm.2007.040071]

2 **Middleton KK**, Barro V, Muller B, Terada S, Fu FH. Evaluation of the effects of platelet-rich plasma (PRP) therapy involved in the healing of sports-related soft tissue injuries. *Iowa Orthop J* 2012; **32**: 150-163 [PMID: 23576936]

3 **Man D**, Plosker H, Winland-Brown JE. The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery. *Plast Reconstr Surg* 2001; **107**: 229-37; discussion 238 [PMID: 11176628 DOI: 10.1097/00006534-200101000-00037]

4 **Tietze DC**, Geissler K, Borchers J. The effects of platelet-rich plasma in the treatment of large-joint osteoarthritis: a systematic review. *Phys Sportsmed* 2014; **42**: 27-37 [PMID: 24875970 DOI: 10.3810/psm.2014.05.2055]

5 **Dhillon RS**, Schwarz EM, Maloney MD. Platelet-rich plasma therapy - future or trend? *Arthritis Res Ther* 2012; **14**: 219 [PMID: 22894643 DOI: 10.1186/ar3914]

6 **Sánchez-González DJ**, Méndez-Bolaina E, Trejo-Bahena NI. Platelet-rich plasma peptides: key for regeneration. *Int J Pept* 2012; **2012**: 532519 [PMID: 22518192 DOI: 10.1155/2012/532519]

7 **Roh YH**, Kim W, Park KU, Oh JH. Cytokine-release kinetics of platelet-rich plasma according to various activation protocols. *Bone Joint Res* 2016; **5**: 37-45 [PMID: 26862077 DOI: 10.1302/2046-3758.52.2000540]

8 **Kobayashi Y**, Saita Y, Nishio H, Ikeda H, Takazawa Y, Nagao M, Takaku T, Komatsu N, Kaneko K. Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations. *J Orthop Sci* 2016; **21**: 683-689 [PMID: 27503185 DOI: 10.1016/j.jos.2016.07.009]

9 **Degen RM**, Conti MS, Camp CL, Altchek DW, Dines JS, Werner BC. Epidemiology and Disease Burden of Lateral Epicondylitis in the United States: Analysis of 85,318 Patients. *HSS J* 2018; **14**: 9-14 [PMID: 29398988 DOI: 10.1007/s11420-017-9559-3]

10 **Oh JH**, Kim W, Park KU, Roh YH. Comparison of the Cellular Composition and Cytokine-Release Kinetics of Various Platelet-Rich Plasma Preparations. *Am J Sports Med* 2015; **43**: 3062-3070 [PMID: 26473014 DOI: 10.1177/0363546515608481]

11 **Weibrich G**, Kleis WK, Hafner G, Hitzler WE. Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count. *J Craniomaxillofac Surg* 2002; **30**: 97-102 [PMID: 12069512 DOI: 10.1054/jcms.2002.0285]

12 **Werner S**, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* 2003; **83**: 835-870 [PMID: 12843410 DOI: 10.1152/physrev.2003.83.3.835]

13 **Wasterlain AS**, Braun HJ, Dragoo JL. Contents and Formulations of Platelet-Rich Plasma. *Oper Tech Orthop* 2012; **22**: 33-42 [DOI: 10.1053/j.oto.2011.11.001]

14 **Monto RR**. Platelet rich plasma treatment for chronic Achilles tendinosis. *Foot Ankle Int* 2012; **33**: 379-385 [PMID: 22735279 DOI: 10.3113/FAI.2012.0379]

15 **Suthar M**, Gupta S, Bukhari S, Ponemone V. Treatment of chronic non-healing ulcers using autologous platelet rich plasma: a case series. *J Biomed Sci* 2017; **24**: 16 [PMID: 28241824 DOI: 10.1186/s12929-017-0324-1]

16 **Mi B**, Liu G, Zhou W, Lv H, Liu Y, Wu Q, Liu J. Platelet rich plasma *vs* steroid on lateral epicondylitis: meta-analysis of randomized clinical trials. *Phys Sportsmed* 2017; **45**: 97-104 [PMID: 28276980 DOI: 10.1080/00913847.2017.1297670]

17 **Shen L**, Yuan T, Chen S, Xie X, Zhang C. The temporal effect of platelet-rich plasma on pain and physical function in the treatment of knee osteoarthritis: systematic review and meta-analysis of randomized controlled trials. *J Orthop Surg Res* 2017; **12**: 16 [PMID: 28115016 DOI: 10.1186/s13018-017-0521-3]

18 **Lisi C**, Perotti C, Scudeller L, Sammarchi L, Dametti F, Musella V, Di Natali G. Treatment of knee osteoarthritis: platelet-derived growth factors vs. hyaluronic acid. A randomized controlled trial. *Clin Rehabil* 2018; **32**: 330-339 [PMID: 28783969 DOI: 10.1177/0269215517724193]

19 **Meheux CJ**, McCulloch PC, Lintner DM, Varner KE, Harris JD. Efficacy of Intra-articular Platelet-Rich Plasma Injections in Knee Osteoarthritis: A Systematic Review. *Arthroscopy* 2016; **32**: 495-505 [PMID: 26432430 DOI: 10.1016/j.arthro.2015.08.005]

20 **Kushida S**, Kakudo N, Morimoto N, Hara T, Ogawa T, Mitsui T, Kusumoto K. Platelet and growth factor concentrations in activated platelet-rich plasma: a comparison of seven commercial separation systems. *J Artif Organs* 2014; **17**: 186-192 [PMID: 24748436 DOI: 10.1007/s10047-014-0761-5]

21 **DeLong JM**, Russell RP, Mazzocca AD. Platelet-rich plasma: the PAW classification system. *Arthroscopy* 2012; **28**: 998-1009 [PMID: 22738751 DOI: 10.1016/j.arthro.2012.04.148]

22 **Graziani F**, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The *in vitro* effect of different PRP concentrations on osteoblasts and fibroblasts. *Clin Oral Implants Res* 2006; **17**: 212-219 [PMID: 16584418 DOI: 10.1111/j.1600-0501.2005.01203.x]

23 **Magalon J**, Bausset O, Serratrice N, Giraudo L, Aboudou H, Veran J, Magalon G, Dignat-Georges F, Sabatier F. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. *Arthroscopy* 2014; **30**: 629-638 [PMID: 24725317 DOI: 10.1016/j.arthro.2014.02.020]

24 **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**: 266-271 [PMID: 21051428 DOI: 10.1177/0363546510387517]

25 **Zimmermann R**, Arnold D, Strasser E, Ringwald J, Schlegel A, Wiltfang J, Eckstein R. Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. *Vox Sang* 2003; **85**: 283-289 [PMID: 14633254 DOI: 10.1111/j.0042-9007.2003.00361.x]

26 **Dejnek M**, Moreira H, Płaczkowska S, Morasiewicz P, Barg E, Witkowski J, Reichert P. Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study. *Adv Clin Exp Med* 2021; **30**: 757-764 [PMID: 34118138 DOI: 10.17219/acem/135045]

27 **Murray IR**, Geeslin AG, Goudie EB, Petrigliano FA, LaPrade RF. Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO): Platelet-Rich Plasma and Mesenchymal Stem Cells. *J Bone Joint Surg Am* 2017; **99**: 809-819 [PMID: 28509821 DOI: 10.2106/JBJS.16.00793]

28 **Mazzucco L**, Balbo V, Cattana E, Guaschino R, Borzini P. Not every PRP-gel is born equal. Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet, RegenPRP-Kit, Plateltex and one manual procedure. *Vox Sang* 2009; **97**: 110-118 [PMID: 19392780 DOI: 10.1111/j.1423-0410.2009.01188.x]

29 **Marx RE**. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent* 2001; **10**: 225-228 [PMID: 11813662 DOI: 10.1097/00008505-200110000-00002]

30 **Mazzocca AD**, McCarthy MB, Chowaniec DM, Cote MP, Romeo AA, Bradley JP, Arciero RA, Beitzel K. Platelet-rich plasma differs according to preparation method and human variability. *J Bone Joint Surg Am* 2012; **94**: 308-316 [PMID: 22336969 DOI: 10.2106/JBJS.K.00430]

31 **Sundman EA**, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med* 2011; **39**: 2135-2140 [PMID: 21846925 DOI: 10.1177/0363546511417792]

32 **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**: e143(1-e1438) [PMID: 23032594 DOI: 10.2106/JBJS.L.00019]

33 **Cieślik-Bielecka A**, Bold T, Ziółkowski G, Pierchała M, Królikowska A, Reichert P. Antibacterial Activity of Leukocyte- and Platelet-Rich Plasma: An *In Vitro* Study. *Biomed Res Int* 2018; **2018**: 9471723 [PMID: 30050949 DOI: 10.1155/2018/9471723]

34 **Dohan Ehrenfest DM**, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 2009; **27**: 158-167 [PMID: 19187989 DOI: 10.1016/j.tibtech.2008.11.009]

35 **Everts PA**, Brown Mahoney C, Hoffmann JJ, Schönberger JP, Box HA, van Zundert A, Knape JT. Platelet-rich plasma preparation using three devices: implications for platelet activation and platelet growth factor release. *Growth Factors* 2006; **24**: 165-171 [PMID: 17079200 DOI: 10.1080/08977190600821327]

36 **Pignatelli P**, Pulcinelli FM, Ciatti F, Pesciotti M, Sebastiani S, Ferroni P, Gazzaniga PP. Acid citrate dextrose (ACD) formula A as a new anticoagulant in the measurement of *in vitro* platelet aggregation. *J Clin Lab Anal* 1995; **9**: 138-140 [PMID: 7714666 DOI: 10.1002/jcla.1860090211]

37 **Bausset O**, Giraudo L, Veran J, Magalon J, Coudreuse JM, Magalon G, Dubois C, Serratrice N, Dignat-George F, Sabatier F. Formulation and storage of platelet-rich plasma homemade product. *Biores Open Access* 2012; **1**: 115-123 [PMID: 23516671 DOI: 10.1089/biores.2012.0225]

Supplementary Table 1 Concentrations (pg/ml) of growth factors and inflammatory cytokines in PRP samples.

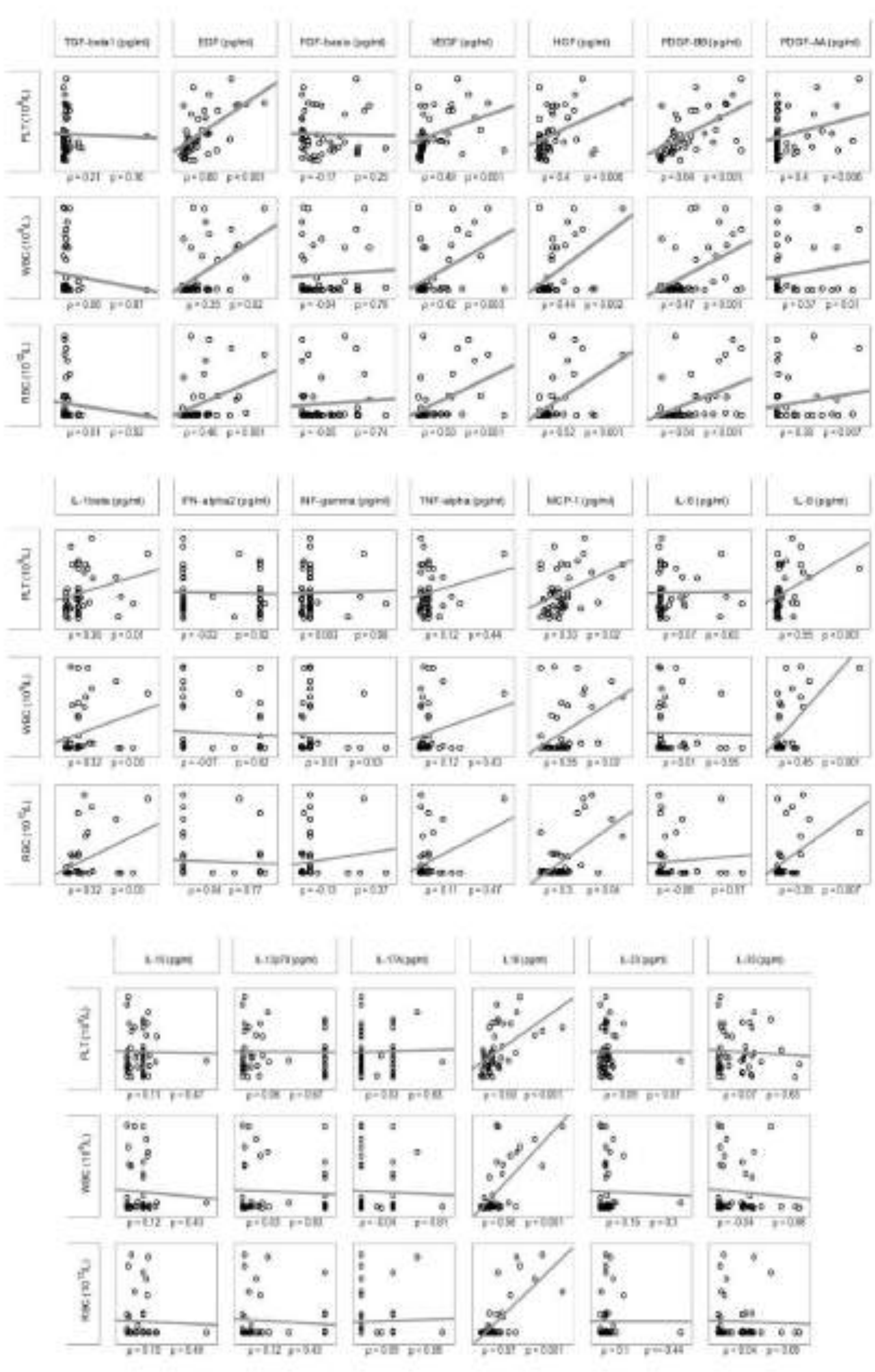
	Arthrex ACP	Mini GPS III	Xerthra	Dr.PRP
EGF	88.61 ± 87.12	384.46 ± 274.27	160.91 ± 215.12	210.64 ± 142.32
FGF-basic	144.16 ± 76.77	109.13 ± 79.42	93.88 ± 61.74	81.90 ± 48.54
HGF	190.80 ± 318.46	590.31 ± 456.27	242.56 ± 329.49	207.16 ± 227.17
VEGF	89.51 ± 149.80	415.21 ± 307.26	178.56 ± 296.15	139.78 ± 128.80
TGF-β1	54.32 ± 69.28	32.93 ± 18.98	34.25 ± 48.42	102.75 ± 259.38
PDGF-AA	26381.73 ± 19002.92	80679.17 ± 38223.38	42018.91 ± 43924.74	43330.41 ± 30601.63
PDGF-BB	7531.75 ± 11740.80	17949.81 ± 26078.98	10575.73 ± 18791.03	12411.06 ± 23248.06
IFN-α2	14.93 ± 4.64	14.68 ± 4.38	14.63 ± 4.34	14.42 ± 4.28
IL-10	10.44 ± 6.09	10.33 ± 5.44	10.67 ± 7.26	13.32 ± 14.09
IL-12p70	12.81 ± 12.51	13.01 ± 12.38	12.70 ± 12.59	13.61 ± 12.42
IL-17A	7.72 ± 0.89	7.64 ± 0.70	7.52 ± 0.53	7.54 ± 0.54
IL18	509.41 ± 353.45	1377.00 ± 908.64	283.01 ± 190.98	414.02 ± 325.62
IL-1β	18.94 ± 21.78	31.08 ± 26.35	17.26 ± 18.45	18.61 ± 18.16
IL-23	10.16 ± 4.97	10.88 ± 7.24	11.20 ± 9.44	15.28 ± 26.19
IL-33	31.52 ± 39.31	27.82 ± 28.36	31.11 ± 37.19	31.84 ± 31.11
IL-6	15.97 ± 24.04	12.72 ± 18.26	12.67 ± 19.12	14.16 ± 19.59
IL-8	139.53 ± 223.56	734.85 ± 864.07	122.98 ± 189.64	168.88 ± 232.11
IFN-γ	21.11 ± 32.10	18.77 ± 23.39	16.94 ± 16.99	18.93 ± 22.29
MCP-1	659.08 ± 336.42	1180.04 ± 834.99	507.43 ± 410.50	625.84 ± 470.53
TNF-α	30.19 ± 26.69	41.38 ± 60.06	21.94 ± 15.17	24.25 ± 21.99

EGF: epidermal growth factor; FGF-basic: fibroblast growth factor-basic; HGF: hepatocyte growth factor; VEGF: vascular endothelial growth factor; TGF-β1: transforming growth factor-β1; PDGF-AA: platelet-derived growth factor-AA; PDGF-BB: platelet-derived growth factor-BB; IFN-α2: interferon-α2; IL-10: interleukin-10; IL-12p70: interleukin-12p70; IL-17A: interleukin-17A; IL18: interleukin-18; IL-1β: interleukin-1β; IL-23: interleukin-23; IL-33: interleukin-33; Il-6: interleukin-6; IL-8: interleukin-8; IFN-γ: interferon-γ; MCP-1: monocyte chemoattractant protein-1; TNF-α: tumor necrosis factor α.

Supplementary Table 2 Significant Spearman correlations between blood cell components and growth factors or cytokines in PRP

Correlation	ρ	P
Arthrex ACP		
PLT (10^9/L) & PDGF-AA (pg/ml)	ρ = 0.58,	P = 0.048
PLT (10^9/L) & INF- γ (pg/ml)	ρ = 0.663,	P = 0.019
PLT (10^9/L) & IL-8 (pg/ml)	ρ = 0.608,	P = 0.036
Mini GPS III		
RBC (10^{12}/L) & IL-1β (pg/ml)	ρ = 0.733,	P = 0.007
RBC (10^{12}/L) & IL-18 (pg/ml)	ρ = 0.615,	P = 0.033
Xerthra		
PLT (10^9/L) & EGF (pg/ml)	ρ = 0.682,	P = 0.021
PLT (10^9/L) & VEGF (pg/ml)	ρ = 0.691,	P = 0.019
PLT (10^9/L) & PDGF-AA (pg/ml)	ρ = 0.773,	P = 0.005
PLT (10^9/L) & IL18 (pg/ml)	ρ = 0.791,	P = 0.004
WBC (10^9/L) & TGF-beta1 (pg/ml)	ρ = 0.738,	P = 0.009
WBC (10^9/L) & EGF (pg/ml)	ρ = 0.836,	P = 0.001
WBC (10^9/L) & VEGF (pg/ml)	ρ = 0.773,	P = 0.005
WBC (10^9/L) & PDGF-AA (pg/ml)	ρ = 0.782,	P = 0.004
RBC (10^{12}/L) & EGF (pg/ml)	ρ = 0.783,	P = 0.004
RBC (10^{12}/L) & VEGF (pg/ml)	ρ = 0.758,	P = 0.007
RBC (10^{12}/L) & PDGF-AA (pg/ml)	ρ = 0.786,	P = 0.004
Dr.PRP		
PLT (10^9/L) & EGF (pg/ml)	ρ = 0.65,	P = 0.022
PLT (10^9/L) & FGF-basic (pg/ml)	ρ = - 0.607,	P = 0.036
WBC (10^9/L) & IL18 (pg/ml)	ρ = 0.833,	P < 0.001
WBC (10^9/L) & IL-23 (pg/ml)	ρ = 0.67,	P = 0.017

EGF: epidermal growth factor; FGF-basic: fibroblast growth factor-basic; VEGF: vascular endothelial growth factor; TGF-β1: transforming growth factor-β1; PDGF-AA: platelet-derived growth factor-AA; IL18: interleukin-18; IL-1β: interleukin-1β; IL-23: interleukin-23; IL-8: interleukin-8; IFN-γ: interferon-γ



Supplementary Figure 1 Spearman correlations between blood cell components and growth factors/inflammatory cytokines in PRP.

V. PRACA NR 3:

Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses

Hindawi
Oxidative Medicine and Cellular Longevity
Volume 2022, Article ID 8059622, 10 pages
https://doi.org/10.1155/2022/8059622

Research Article

Leukocyte-Rich Platelet-Rich Plasma as an Effective Source of Molecules That Modulate Local Immune and Inflammatory Cell Responses

Maciej Dejnek[1], **Helena Moreira**[2], **Sylwia Płaczkowska**[3], **Ewa Barg**[2], **Paweł Reichert**[1], and **Aleksandra Królikowska**[4]

[1]*Clinical Department of Trauma and Hand Surgery, Department of Trauma Surgery, Faculty of Medicine, Wroclaw Medical University, 50-556 Wroclaw, Poland*
[2]*Department of Medical Science Foundation, Faculty of Pharmacy, Wroclaw Medical University, 50-556, Wroclaw, Poland*
[3]*Teaching and Research Diagnostic Laboratory, Department of Laboratory Diagnostics, Faculty of Pharmacy, Wroclaw Medical University, 50-556 Wroclaw, Poland*
[4]*Ergonomics and Biomedical Monitoring Laboratory, Department of Physiotherapy, Faculty of Health Sciences, Wroclaw Medical University, 50-355 Wroclaw, Poland*

Correspondence should be addressed to Maciej Dejnek; maciej.dejnek@student.umw.edu.pl

Received 4 May 2022; Accepted 18 July 2022; Published 2 August 2022

Academic Editor: Ana Lloret

Copyright © 2022 Maciej Dejnek et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Autologous platelet-rich plasma (PRP) injection is a safe biological method used to treat various musculoskeletal diseases. By downregulation of inflammatory cytokines and stimulation of synovial fibroblasts, PRP injection is a promising adjunctive treatment for patients with chronic autoimmune inflammatory diseases such as rheumatoid arthritis. A major problem in comparing the results of clinical trials in this area is the considerable variability in the cytokine content of PRP. We presented the profile of selected growth factors and inflammatory cytokines in the obtained PRP samples and compared them with baseline serum levels to assess the efficacy of PRP as a source of those paracrine molecules. Additionally, we wanted to determine whether the difference is only quantitative, which would suggest the use of a cheaper alternative by injecting a large amount of autologous serum. For this purpose, we analyzed whole blood and PRP samples prepared using the Mini GPS III Platelet Concentration System (Biomet Inc., USA) in 31 subjects aged 35-60 years. Cellular content, seven selected growth factors, and 13 human inflammatory cytokines were evaluated. Multiplex bead immunoassays that use fluorescence-encoded beads LEGENDplex™ (BioLegend, USA) and flow cytometer measurements were used. As a result, we found a statistically significant increase in four of the growth factors tested and eight of the inflammatory cytokines tested in PRP compared to blood serum. The difference is not only quantitative but also in the composition of paracrine molecules. In conclusion, the study confirmed that PRP is an efficient source of several growth factors and some inflammatory cytokines. These data provide additional insight into the potential mechanisms of PRP's effects on cellular metabolism and inflammatory response and may contribute to a better understanding of its clinical efficacy.

1. Introduction

Autologous platelet-rich plasma (PRP) injection is a treatment method used in various soft tissue degenerative conditions. It is widely used in orthopedic, sports medicine, stomatology, and aesthetic medicine [1–3]. A growing number of reports indicate a potential role for PRP in the treatment of involved joints in patients with chronic autoimmune inflammatory diseases such as rheumatoid arthritis [4, 5].

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that affects approximately 1% of the world's population and is characterized by synovial hyperplasia, articular inflammation, and invasion of the synovium

into the adjacent bone and cartilage [6]. The underlying cause of the disease is an abnormal immune response that activates fibroblast-like synoviocytes (FLS), provoking an inflammatory response that leads to disease progression [4]. In the initial stage, the disease usually affects small joints, but when advanced, it can lead to massive destruction of large joints such as the knee, shoulder, or hip, causing significant disability. The exact cause of the disease is not sufficiently understood, although many different therapies targeting the molecular pathways of the ongoing inflammatory process have been proposed [4]. One of the new methods supporting the treatment of patients is the intra-articular injection of PRP. Studies have shown high safety of the therapy resulting in suppression of the inflammatory process [4, 5, 7].

PRP is obtained by the separation of plasma with an excessive amount of platelets from the blood sample. The most common preparation method is manually extracting platelet-rich plasma from the blood sample after centrifugation in a specially designed tube [8]. Many different commercially available kits existing on the market are helping to prepare PRP as easily as possible in ambulatory conditions.

This treatment is aimed at providing a high amount of platelet-derived growth factors to enhance healing issues and stimulate degenerative tissue to regenerate or modulate the local inflammatory response [9]. In the human body, high amounts of growth factors and cytokines are released from platelets alpha-granules at the site of injury. Their role is to stimulate cell migration, proliferation, differentiation, angiogenesis, extracellular matrix production, and scar formation [10]. An enormous amount of various cytokines affect the local environment and interact with each other through the processes of stimulation and inhibition [9]. The function of many of them remains unclear and is difficult to assess in the living organism.

There are many doubts about the effectiveness of PRP treatment in different conditions. The results presented by various researchers are often mutually exclusive, and most meta-analyses do not provide clear answers in this field [1]. There may be several reasons for this situation. Significant differences in the content of biologically active components in PRPs prepared by different commercially available kits and their repeatability in the platelet and leukocyte concentrations are among the problems to be solved [11]. Furthermore, significant differences due to the patients' characteristics, such as age, sex, smoking status, diseases, drugs usage, or physical activity, can affect the quality of the final PRP product [12–14]. Incorrect interpretation of the results may also be due to the incompletely understood function of all cytokines in the PRP samples and their interaction with each other and the surrounding tissues. Many more studies are still needed both on the content of biologically active components in PRP samples and their influence on the effectiveness of treatment of various diseases.

The aim of the study was to assess if PRP samples would have a significantly higher amount of selected growth factors and inflammatory cytokines than baseline level in patients' serum and if the difference would be only quantitative or qualitative as well. We hypothesized that PRP would contain a proportionally higher concentration of paracrine molecules than patients' serum. Confirmation of the above hypothesis could lead to the development of a new, cheaper orthobiological treatment method by intra-articular injection of a higher volume of autologous serum, which could be beneficial for large joints affected by rheumatoid arthritis.

2. Materials and Methods

2.1. Ethical Standards. The study was carried out according to the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Wroclaw Medical University (KB–26/2019, 21.01.2019). All patients agreed to participate in the study and signed an informed consent.

2.2. Study Design. The study was designed as a single-center prospective descriptive laboratory study and is an additional part of the investigation of the correlation between the concentration of cytokines in PRP and the effectiveness of epicondylopathy treatment registered in clinicaltrials.gov under identifier NCT04521387.

2.3. Population. The study was conducted on 31 patients who were enrolled in the years 2021-2022. The population contained 15 men and 16 women aged 35-60 years old ($x = 49.10$; $SD = 6.03$). Patients' height and weight were measured to calculate Body Mass Index (BMI). Three patients regularly smoked cigarettes, 15 regularly practiced sports activity (≥3 sessions per week), 22 drank alcohol occasionally (≤1 dose per week), and nine did not drink alcohol at all. Patients with hematologic diseases, diabetes, and suspicion of the infectious process; those who are pregnant; and those taking medications that may affect platelet function or the coagulation system were excluded.

2.4. PRP Preparation. From each patient, 27 ml of blood was collected into a 30 ml syringe filled with 3 ml of anticoagulant citrate dextrose solution A (ACD-A). Additional 6 ml of blood was taken and divided to a 2 ml probe with ethylenediaminetetraacetic acid (EDTA) for complete blood count analysis and a 4 ml probe with clotting activator for serum preparation. A 30 ml syringe blood sample mixed with ACD-A was transferred to a specially designed tube with a membrane for PRP separation—Mini GPS III Platelet Concentration System (Biomet Inc., USA). The tube was placed in a dedicated centrifuge for the separation process, which took 15 minutes with 3200 revolutions per minute (RPM). After centrifugation, platelet-poor plasma placed above the separation membrane was removed, and 3 ml liquid-form leukocyte-rich platelet-rich plasma was collected in the sterile 3 ml syringe according to the manufacturer manual. The step-by-step process for preparing PRP is shown in Figure 1. Two ml of PRP was used for patient treatment, and 1 ml of PRP was reinjected into an Eppendorf polypropylene tube and then gently shaken for 30 seconds just before laboratory analysis. The time between blood draw, PRP separation, and further analysis did not exceed 1 hour, and the whole process was conducted in daylight at room temperature.

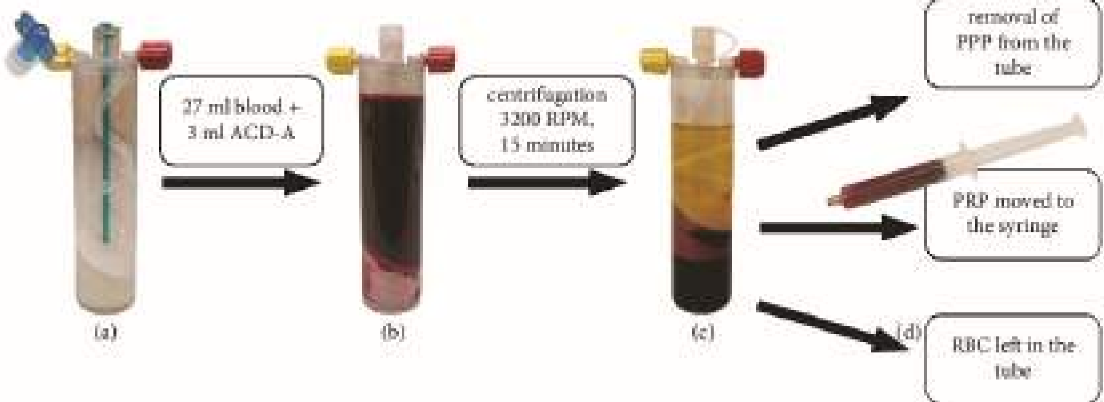


Figure 1: PRP preparation process by Mini GPS III. (a) The empty Mini GPS III tube. (b) The tube filled with 3 ml of anticoagulant citrate dextrose solution A (ACD-A) and 27 ml of patients' own blood. (c) The tube after centrifugation containing three separate layers: platelet-poor plasma (PPP), platelet-rich plasma (PRP), and red blood cells (RBC). (d) The syringe filled with PRP taken from the tube after the removal of PPP.

2.5. Evaluation of Cellular Components. The complete blood count was analyzed from a 2 ml probe with EDTA using the Mindray BC-5150 automatic laboratory analyzer (Shenzhen Mindray Bio-Medical Electronics Co., PRC). Then, the PRP sample was analyzed in the same fashion from the Eppendorf tube.

2.6. Serum Sample Preparation and Storage. Blood was collected into BD Vacutainer® Plus Plastic Serum Tubes (BD, Biosciences, Warsaw, Poland). The blood samples were allowed to clot at room temperature for approximately 20–30 min and centrifuged at 500 × g for 15 minutes. The serum was transferred to a polypropylene tube and stored at -80°C until the time of analysis.

2.7. PRP Activation and Storage. PRP samples were activated through a double freeze-thaw process for 30 minutes in each step as proposed by Zimmermann et al. [15]. The activated samples were frozen to -80°C and stored for further analysis.

2.8. Growth Factor and Inflammatory Cytokine Evaluation. LEGENDplex™ Custom Human 7-plex Panel and LEGENDplex™ Human Inflammation Panel 1 (BioLegend, USA) were used to estimate the concentration of selected growth factors and inflammatory cytokines in PRP and serum samples. The first one is a customized panel dedicated to our study, containing the most frequently studied and the most important platelet-derived growth factors. The second one is a commercially available standard set for inflammatory cytokine testing (see Table 1). LEGENDplex is a multiplex immunoassays based on fluorescence-encoded beads and flow cytometric measurements. Just before the assay, all samples were thawed to room temperature, centrifuged for 5 minutes at 2500 RPM in a Micro Star 17 microcentrifuge (VWR International Company, Thermo Electron LED, Germany), and 2x diluted. A LEGENDplex assay was performed according to the manufacturer's procedure. The samples were acquired on a CyFlow Cube 8 flow cytometer (Sysmex-Partec, Görlitz, Germany) applying a 488 nm laser with 536/40 (BP) filter for the PE fluorochrome and a 638 nm laser with 675/20 (BP) for the APC fluorochrome. The results were analyzed using LEGENDplex™ Data Analysis Software V.8.0 (Vigene Tech Inc., USA). The concentration of each growth factor/cytokine was determined by means of a standard curve generated during the performance of the assay.

2.9. Statistical Analysis. For statistical assessment, Statistica 13.3 software (TIBCO Software Inc, USA) was used. For compliance of the result distribution, the Shapiro-Wilk test was used. Arithmetic means and standard deviations (SD) were calculated. For data with nonnormal distribution, the median and quartile distribution (Q1-Q3) were additionally given. The outliers (more than 3 standard deviations) were removed for the calculations. For comparison of two variables with normal distribution, dependent t-test for paired samples was performed. For variables without normal distribution, the Wilcoxon signed rank test was performed. Pearson's correlation coefficient (r) was used to establish a relationship between blood cell components and growth factors or inflammatory cytokines. The level equal to or greater than 0.8 was assumed to be the satisfactory power of the tests ($1 - \beta > 0.8$). Based on an earlier pilot study, we calculated that 30 patients would be sufficient to achieve the power target for the growth factor comparison [11]. The results were assessed as significant at $p \leq 0.05$.

3. Results

3.1. Cellular Components. Differences between whole blood and PRP samples in cellular content are highlighted in Table 2. All differences except the content of eosinophils ($p = 0.52$) were significant ($p \leq 0.001$). WBC and PLT but not RBC content in whole blood correlate ($r > 0.30$) to their concentrations in PRP. There was significant difference between men and women in RBC content in whole blood (5.15 *vs.* 4.56, $p \leq 0.001$), which is a physiological difference within the normal range for both sexes. WBC content in PRP also differed between males and females (34.64 *vs.* 25.83, $p < 0.05$). Current smoking status was connected with

Table 1: All paracrine molecules tested by flow cytometry with the use of LEGENDplex™ panels.

LEGENDplex™ Custom Human 7-plex Panel	LEGENDplex™ Human Inflammation Panel 1
Transforming growth factor-β1 (TGF-β1, free active)	Interleukin-1β (IL-1β)
Epidermal growth factor (EGF)	Interferon-α2 (IFN-α2)
Fibroblast growth factor-basic (FGF-basic)	Interferon-γ (IFN-γ)
Vascular endothelial growth factor (VEGF)	Tumor necrosis factor α (TNF-α)
Hepatocyte growth factor (HGF)	Monocyte chemoattractant Protein-1 (MCP-1)
Platelet-derived growth factor-AA (PDGF-AA)	Interleukin-6 (IL-6)
Platelet-derived growth factor-BB (PDGF-BB)	Interleukin-8 (IL-8)
	Interleukin-10 (IL-10)
	Interleukin-12p70 (IL-12p70)
	Interleukin-17A (IL-17A)
	Interleukin-18 (IL-18)
	Interleukin-23 (IL-23)
	Interleukin-33 (IL-33)

Table 2: Differences in cellular content between whole blood (WB) and platelet-rich plasma (PRP). Values are presented as arithmetic mean (standard deviation). The ability to concentrate the cell components in PRP vs. WB is presented as the "ratio." The Pearson correlation coefficient between the cell components of WB and PRP is presented as a value r ($^*p < 0.050$). The significance of the comparison is shown as p value.

	WB	PRP	Ratio	p	r
WBC ($10^3/\mu l$)	6.60 (1.34)	30.09 (9.58)	×4.59	≤0.001	0.66*
Neutrophils	4.03 (1.22)	12.72 (6.66)	×3.19	≤0.001	0.47*
Lymphocytes	1.96 (0.51)	14.18 (5.04)	×7.31	≤0.001	0.64*
Monocytes	0.4 (0.1)	2.81 (1.07)	×7.25	≤0.001	0.73*
Eosinophils	0.15 (0.11)	0.17 (0.17)	×1.06	0.52	0.78*
Basophiles	0.03 (0.02)	0.19 (0.12)	×6.44	≤0.001	0.85*
RBC ($10^6/\mu l$)	4.85 (0.44)	0.92 (0.49)	×0.19	≤0.001	0.14
PLT ($10^3/\mu l$)	253.27 (59.37)	1083.87 (493.55)	×4.41	≤0.001	0.47*

a significantly higher concentration of PLT in whole blood and PRP ($p < 0.05$). Alcohol consumption and sports activity did not influence the cellular content of the whole blood and PRP.

3.2. Growth Factors. The differences between the serum and PRP growth factor content are presented in Table 3. All growth factors increased in PRP, but the difference was not significant for VEGF. Sex did not influence any of the growth factors. Smokers had significantly higher concentration of PDGF-BB (70486.93 *vs.* 46856.9, $p < 0.05$) which could be explained by a higher PLT content in their whole blood and PRP samples. Alcohol consumption and sports activity did not influence growth factor content in the whole blood and PRP.

3.3. Inflammatory Cytokines. Differences between serum and PRP inflammatory cytokine content are highlighted in Table 4. All inflammatory cytokine concentrations in the PRP were lower in women, and these differences were significant ($p < 0.05$) in all except MCP-1 ($p = 0.63$), IFN-γ ($p = 0.10$), TNF-α ($p = 0.12$), and IL-6 ($p = 0.27$). Comparison between both sexes is shown in Supplementary Table 1. Smoking status, alcohol consumption, and sports activity did not influence the content of cytokines in the PRP samples. More than 80% of the IL-23 results were below the lower cut-off point for measurement; therefore, it was excluded.

3.4. Correlations between Cell Content and Growth Factors or Inflammatory Cytokines. Significant high positive correlation was found between PLT content in PRP and three growth factors: EGF ($r = 0.74$; $p \leq 0.001$), PDGF-AA ($r = 0.77$; $p \leq 0.001$), and PDGF-BB ($r = 0.79$; $p \leq 0.001$). Significant but low positive correlation was found between PLT and VEGF ($r = 0.46$; $p < 0.05$) (see Figure 2). No significant correlations between PLT content and inflammatory cytokines were observed.

Significant moderate positive correlation was found between WBC and VEGF ($r = 0.69$; $p \leq 0.001$), and significant low correlation between WBC and HGF ($r = 0.42$; $p < 0.05$). For inflammatory cytokines, a significant positive low correlation was found between WBC and IL-8 ($r = 0.45$; $p < 0.05$) (see Figure 3). Subpopulation of neutrophils correlated moderately with IL-8 ($r = 0.62$; $p \leq 0.001$) and on a low level with IL-1β ($r = 0.41$; $p < 0.05$) and IL-18

Table 3: Differences in growth factor content between serum and platelet-rich plasma (PRP). Values (pg/ml) are presented as arithmetic mean (standard deviation) and median (Q1-Q3). The ability to concentrate growth factors in PRP vs. serum is presented as the "ratio." The significance of the comparison is shown as p value.

	Serum		PRP		Ratio	p
	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)		
TGF-β1, free active	151.67 (72.41)	158.02 (94.86-204.24)	383.33 (251.75)	343.26 (257.65-443.32)	×3.79	≤0.001
EGF	110.04 (44.88)	103.575 (76.33-140.36)	243.96 (155.21)	219.13 (116.43-319.81)	×2.5	≤0.001
FGF-basic	1094.06 (487.25)	1048.33 (667.71-1436.06)	746.30 (2043.42)	315.30 (254.70-428.24)	×0.68	≤0.001
VEGF	152.88 (52.91)	152.925 (128.87-171.73)	324.53 (394.54)	180.67 (40.95-313.17)	×2.34	0.43
HGF	534.72 (210.44)	497.91 (375.67-679.67)	231.23 (114.08)	204.03 (156.05-258.75)	×0.51	≤0.001
PDGF-AA	25462.54 (13742.23)	23429.45 (16404.90-32176.13)	132725.23 (53608.77)	137269.89 (90062.55-183956.91)	×6.19	≤0.001
PDGF-BB	9070.09 (6484.37)	6807.38 (5424.22-13513.71)	49143.68 (18068.74)	51779.04 (29826.86-62573.63)	×6.43	≤0.001

Table 4: Differences in inflammatory cytokine content between serum and platelet-rich plasma (PRP). Values (pg/ml) are presented as arithmetic mean (standard deviation) and median (Q1-Q3). The ability to concentrate the cytokines in PRP vs. serum is shown as the "ratio." The significance of the comparison is presented as p value.

	Serum		PRP		Ratio	p
	Mean (SD)	Median (Q1-Q3)	Mean (SD)	Median (Q1-Q3)		
IL-1β	34.09 (48.04)	18.12 (18.12-19.78)	67.09 (58.67)	43.24 (30.36-82.03)	×2.99	0.002
IFN-α2	16.90 (2.54)	16.16 (16.16-16.16)	39.0 (28.68)	26.38 (16.16-50.14)	×2.40	≤0.001
IFN-γ	4.98 (0.56)	4.87 (4.87-4.87)	6.66 (3.09)	4.87 (4.87-7.13)	×1.39	0.03
TNF-α	18.50 (7.45)	13.17 (13.02-20.98)	33.1 (27.09)	23.56 (13.02-42.72)	×2.12	0.02
MCP-1	140.22 (162.49)	80.3 (30.82-194.90)	107.90 (66.07)	88.71 (63.97-148.27)	×2.13	0.99
IL-6	13.14 (2.61)	12.16 (12.16-12.50)	18.70 (9.77)	14.64 (12.16-22.04)	×1.52	0.009
IL-8	47.45 (96.35)	16.39 (12.14-33.46)	125.99 (131.44)	64.38 (46.77-157.73)	×5.89	≤0.001
IL-10	13.62 (4.09)	11.76 (11.76-12.59)	18.84 (8.12)	15.81 (11.76-23.69)	×1.46	0.007
IL-12p70	13.89 (4.22)	12.01 (10.66-17.21)	20.75 (11.30)	16.12 (10.66-27.8)	×1.62	0.01
IL-17A	2.50 (0.87)	1.94 (1.94-2.85)	3.18 (1.69)	2.56 (1.94-4.28)	×1.41	0.15
IL-18	251.82 (167.62)	189.23 (151.49-308.10)	403.01 (292.19)	323.65 (183.26-507.57)	×2.45	0.14
IL-33	117.58 (46.18)	101.795 (83.24-143.64)	176.58 (99.23)	159.09 (95.95-229.86)	×1.75	0.11

($r = 0.48$; $p < 0.05$). Subpopulation of lymphocytes correlated moderately with FGF-basic ($r = 0.53$; $p < 0.05$) and on a low level with EGF ($r = 0.43$; $p < 0.05$) and VEGF ($r = 0.48$; $p < 0.05$).

Age and BMI did not influence the content of PLT, WBC, RBC, and all growth factors in PRP. Among inflammatory cytokines, age significantly negatively correlates with IL-1β ($r = -0.50$; $p < 0.05$), IFN-α2 ($r = -0.38$; $p < 0.05$), and TNF-α ($r = -0.39$; $p < 0.05$). BMI was positively correlated only with IL-1β ($r = 0.40$; $p < 0.05$). However, these correlations were on a low level.

4. Discussion

We hypothesized that PRP prepared with Mini GPS III Platelet Concentration System would contain a proportionally higher concentration of paracrine molecules than patients' serum. The results show that PRP delivers higher doses of TGF-β1 free active, EGF, VEGF, PDGF-AA, and PDGF-BB but not HGF and FGF-basic. However, the profile of inflammatory cytokines differed significantly only for eight from thirteen measured: IL-1β, IFN-α2, IFN-γ, TNF-α, IL-6, IL-8, IL-10, and IL-12p70. These differences cannot be simply explained by a higher concentration of platelets or white blood cells. Increases in platelet-derived growth factor concentration (PDGF-AA, PDGF-BB) and TGF-β1 were closest to the achieved increase in platelet concentration (×6.19, ×6.43, and 3.79, respectively, vs. ×4.41). The two other growth factors increased more than two times (VEGF × 2.34, EGF × 2.5) and the other two decreased. Those inflammatory cytokines, the concentration of which increased significantly, increased from 1.39 to 5.89. This leads to the assumption that in PRP, we obtain a different cytokine profile, not just a quantitative increase in concentration. There is a risk of bias due to the serum preparation protocol, which requires cloth formation. Attention should be paid to this critical detail, as it can directly impact the clinical effectiveness of the proposed treatment.

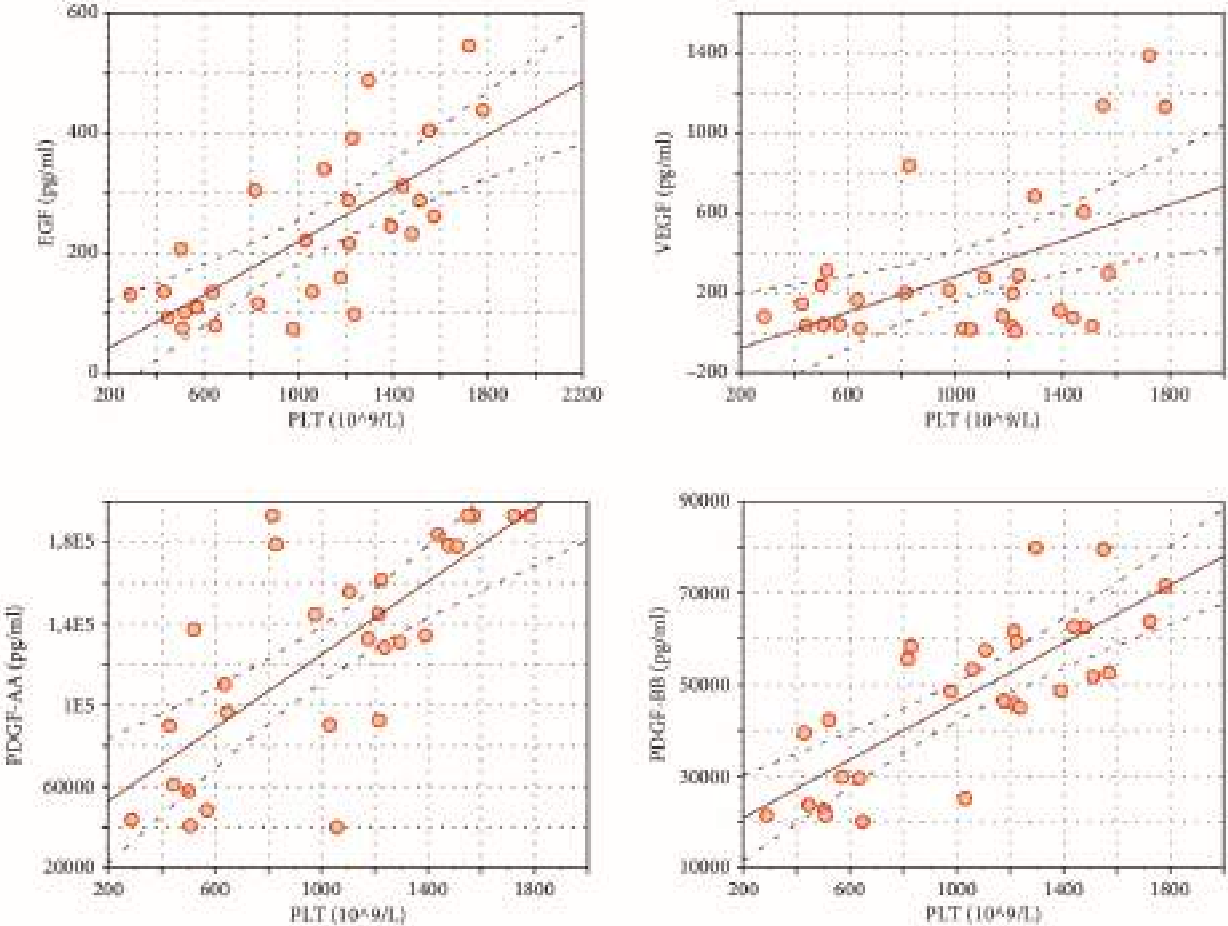


Figure 2: Significant ($p < 0.05$) Pearson correlation coefficient (r) between platelets (PLT) and growth factors: EGF ($r = 0.74$), VEGF ($r = 0.46$), PDGF-AA ($r = 0.77$), and PDGF-BB ($r = 0.79$).

Multiple protocols have been developed for PRP preparation. Many commercially available techniques differ in the shape of the separator used; the amount of blood collected and PRP obtained; the amount, time, and force of centrifugation; and the separation technique. As a result, they differ in the concentration of platelets, leukocytes, and red cell contamination obtained. These differences significantly affect the content of biologically active molecules that may have a therapeutic effect [8, 11]. Different divisions have been proposed to facilitate the comparison of PRP treatment results. The most commonly used takes into account fibrin and leukocyte content, dividing PRP into four categories: leukocyte-rich or pure platelet-rich plasma (L-PRP, P-PRP) and leukocyte-rich (L-PRF) or pure platelet-rich fibrin (P-PRF) [16]. Additionally, it is worth distinguishing systems enabling high (5-9×) and low (2.5-3×) concentrations of platelets [17]. Currently, the most complex classification system was proposed by Lana et al. [18]. The system called MARSPILL includes interalia, specifying the number of platelets, white blood cells, red blood cells, spins, activation process, and image guidance during PRP administration. The above classification perfectly illustrates the complexity of assessing the effectiveness of different PRP treatments.

Most researchers define PRP as plasma with platelet concentrations above 1 million per microliter [19, 20]. Commercially available systems do not always meet this criterion. The US Food and Drug Administration (FDA) allows the term PRP to be applied to products that have platelet concentrations above 250,000 per microliter. In addition, Mazzucco et al.'s work concluded that platelet concentrations above 200,000 per microliter are sufficient for a therapeutic effect [21]. This can lead to confusion as this platelet count is within the normal range for whole blood, and it is hard to expect a higher density of active cytokines in it. In our work, we used the Mini GPS III Platelet Concentration System, one of the most widely used PRP preparation systems globally. It allows obtaining reproducible results with 4-5 times platelet density and higher than baseline concentration of white blood cells [11].

The WBC content of the obtained PRP can influence the concentration of various cytokines and modulate local immune and inflammatory responses. Both negative and positive effects of leukocyte-rich PRP on tissue healing have been reported in the literature. Threats arise from the potential catabolic effects of leukocytes on surrounding tissues through the release of proinflammatory cytokines

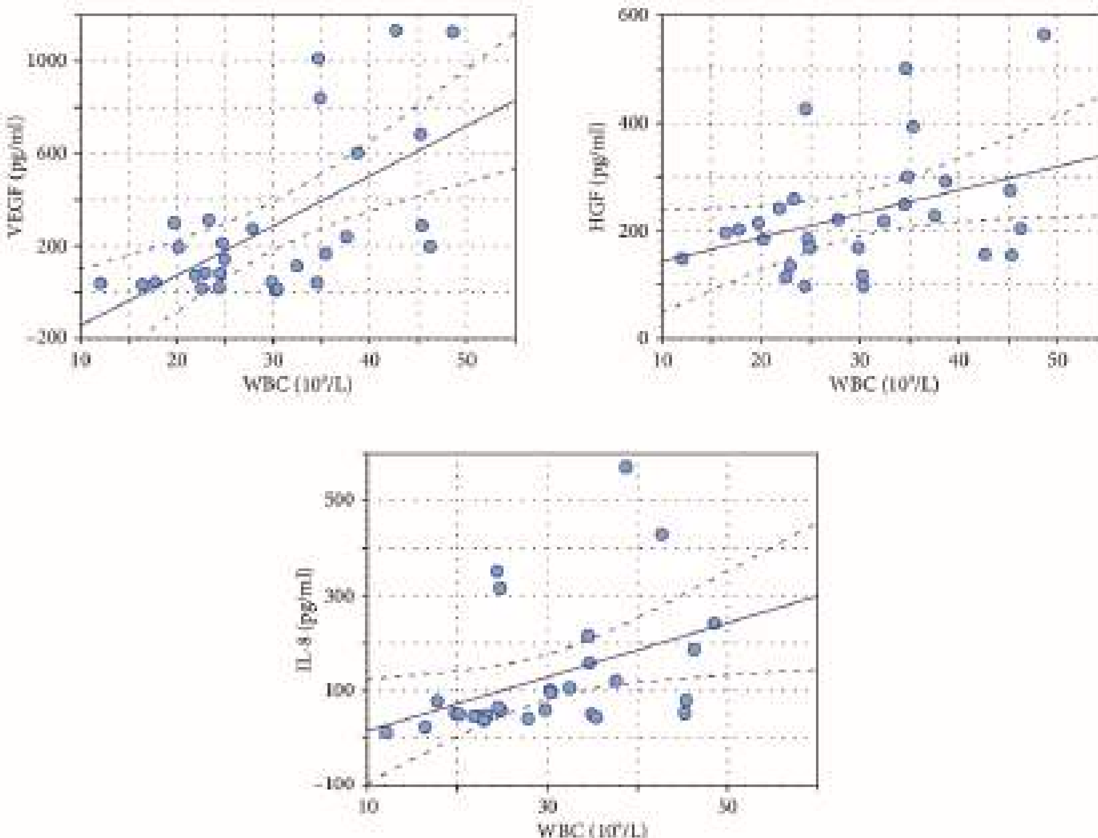


Figure 3: Significant ($p < 0.05$) Pearson correlation coefficient (r) between white blood cells (WBC) and growth factors/cytokines: VEGF ($r = 0.69$), HGF ($r = 0.42$), and IL-8 ($r = 0.45$).

and proteinases. The benefits of a high WBC content are associated with positive correlations with certain growth factors, the ability of leukocytes to modulate immune responses, antimicrobial properties, and reported favourable clinical outcomes [22–25].

Previous studies have shown correlations between some cytokines and cell contents in PRP, but only a few have compared their contents and those in whole blood [25, 26]. A significant positive correlation was found between PLT content in PRP and EGF, VEGF, PDGF-AA, and PDGF-BB in these studies. Magalon et al. evaluated the content of EGF, VEGF, TGF-β1, and PDGF-AB in PRP obtained with Mini GPS III. The PLT and WBC concentrations were similar to our findings, but the growth factor content was much higher for VEGF and EGF (×3.2 and ×9.3, respectively) than in our study. The concentration of the representative of PDGF family in their research (PDGF-AB) was much lower than that of PDGF-AA or PDGF-BB in our study [27]. The possible reason for these differences is the addition of calcium chloride for platelet activation. The double freeze-thaw process activates platelets in a more physiological way, which is why it was used in our study [15]. Similar to our finding, they presented a significant positive correlation between EGF, VEGF, PDGF, and PLT concentration in PRP. Contrary to our results, they also found a correlation between PLT and TGF-β1 [27]. The concentration of WBC in PRP correlates with the content of VEGF and EGF in their study. Our findings support only the correlation between WBC and VEGF. Another study reporting growth factor content in PRP obtained by GPS III was carried out by Castillo et al. [28]. The concentration of WBC in the final sample was similar to that presented in our study, but PLT mean concentration was almost two times lower. That could be due to the fact that they used a different version of the GPS III system, which requires approximately 55 ml of blood donation and other centrifugation parameters. They assessed the final concentrations of PDGF-AB, PDGF-BB, TGF-β1, and VEGF. In their results, PDGF-BB was two times lower, but VEGF 6 times higher than those found in our study. Just as a decreased PDGF level can be explained by a lower platelet content, the differences in other factors are difficult to explain. In the literature review, Oudelaar et al. collected seven studies presenting the content of PLT, WBC, PDGF-AB, VEGF, and TGF- β1 in PRP obtained with the GPS III system [8]. The contents of PLT and WBC were similar between the studies and comparable to our results. However, the content of growth factors varied considerably, especially TGF-β1. In most of the mentioned studies, the concentration of VEGF was higher than in our samples. Concentrations of TGF-β1 were much higher because we evaluated the concentration of TGF- β1 free active, whose levels are about 65 times lower than total TGF-β1 in serum [29].

Among the best described is the platelet-derived growth factor family (PDGF) role in the healing process both in vitro, in animal models, and in patients with wound healing disorders. It stimulates neutrophils, monocytes, and fibroblasts to migrate to the wound site and activates the latter mentioned to proliferate and produce an extracellular matrix [9]. The fibroblast growth factor (FGF) family has mitogenic activity and a positive effect on cell migration and differentiation and participates in cytoprotection during stress conditions [10]. Angiogenesis at the wound site is induced and stimulated by vascular endothelial growth factor (VEGF) [9]. Other growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor-β1 (TGF-β1) have an impact on proper cell proliferation and differentiation during the healing process [9, 10, 30].

Among a wide spectrum of tested inflammatory cytokines, a significant positive correlation was found only between WBC and IL-8. This cytokine has some potential positive effects on healing and immunomodulation by its ability to stimulate reepithelialization and to attract neutrophils to the site of injury. In the literature, only a few authors have performed an analysis of inflammatory cytokines in PRP. The concentrations of IL-1β and matrix metalloproteinase-9 (MMP-9) in PRP were presented in two studies [25, 26]. The authors found that MMP-9 and IL-1β levels were much higher in leukocyte-rich PRP and were significantly correlated with neutrophil concentration [25]. Those two are catabolic cytokines known for their role in inflammation and matrix degradation. MMP-9 has been implicated as a predictor of improper healing [26]. We also found significant but low correlation between neutrophil concentration and IL-1β. The healing process is also modulated by a number of different cytokines that can stimulate migration of macrophages like monocyte chemoattractant protein (MCP), stimulate reepithelialization such as interleukin-8 (IL-8), inhibit inflammation and scar formation such as interleukin-10 (IL-10) [10]. A complex process of interaction between numerous anti- and proinflammatory cytokines regulates the course of tissue healing, leading in the most desirable case to recovery.

In our study, age and BMI did not influence the content of PLT, WBC, RBC, and all growth factors in PRP. Dragoo et al., in their study, found a significant negative correlation between age and PDGF-BB. The concentration of PDGF-BB in the PRP was higher in 18-30-year-old subpopulation; however, among the older subpopulations, the values were stable and oscillated about 31 ng/ml [31]. Among inflammatory cytokines in our research, age was significantly negatively correlated with IL-1β, IFN-α2, and TNF-α. The statistically significant differences in the cytokine content in PRP between the sexes are difficult to explain, especially since they were not shown when comparing the serum samples. A possible explanation would be the uneven distribution of cytokines during centrifugation, forced by other components. Solving this problem would require further, more detailed research.

Rheumatoid arthritis is a chronic autoimmune inflammatory disease characterized by progressive destruction of cartilage and bone with periods of acute exacerbation [32]. The correlation of cytokines such as TNF-α, IL-6, and IL-1β with the intensity of the disease has been demonstrated [32]. This is also the reason why TNF-α inhibitors were developed to treat RA. The increased level of VEGF is probably related to the stimulation of neovascularization during the ongoing inflammatory process. In an animal model of arthritis, Lippross et al. found that PRP injection leads to the reduction of IL-6, IL-1, IGF-1, and VEGF in cartilage and synovium. TNF-α did not change after injection of PRP [32]. Tong et al. presented results on a type II collagen-induced arthritis mouse model treated with PRP. The study reported a downregulation of the expression of IL-6, IL-8, IL-17A, IL-1β, TNF-α, receptor activator for nuclear factor-κB, and IFN-γ in inflammatory tissue [7]. They also found that PRP can be beneficial due to decreased joint inflammation, cartilage destruction, bone damage, and increased joint tissue repair [7]. On the other hand, in their papers, both Yan et al. and Wang et al. highlighted the risk of rheumatoid arthritis fibroblast-like synoviocytes cell migration, invasion, and adhesion stimulated by MMP-1, whose expression was increased after PRP administration [6, 33].

Only two studies evaluating PRP efficacy on rheumatoid arthritis have been published. Badsha et al. presented results of PRP injection to the knee joint of four subjects. They reported a significant improvement in the disease activity score, reduced pain, and a decrease of joint inflammation during ultrasound examination [5]. Saif et al. evaluated the therapeutic effect of intra-articular PRP versus steroid in RA patients and their impact on inflammatory cytokines, local joint inflammation, disease activity, and quality of life. In this randomized controlled trial, 60 patients with RA were divided into two equal groups. Both groups showed improvements at 3 months after injection, but only in the PRP group this improvement lasted up to 6 months. Downregulating effects on inflammatory cytokines (IL-1β, TNF-α) with subsequent improvement of local joint inflammation, disease activity, and quality of life were presented in the PRP group. The authors concluded that PRP injections were a safe and valuable treatment option for RA patients [34].

These findings lead us to the hypothesis that intra-articular PRP injections could help patients with RA through two main mechanisms. The first is the positive role of growth factors such as PDGF, EGF, FGF, or TGF-β1 in stimulating healing, regeneration, and protecting articular cartilage. The second is the ability of PRP to downregulate the expression of major inflammatory cytokines such as IL-1β, IL-6, and TNF-α leading to a reduction in local inflammation. The exact molecular pathway of the PRP interaction will be challenging to determine due to the multitude of biologically active ingredients it contains.

There are some limitations of the presented study. The major limitation is that we evaluated only one particular PRP preparation protocol, which, due to its distinctiveness, may in itself influence the final cytokine profile. Some of the existing numerous cytokines that may affect the local cellular response have been omitted for technical reasons. The direction of further research should be to compare the

biological effect in vitro or in vivo depending on the profile of biologically active components in PRP.

5. Conclusions

The study showed that autologous leukocyte-rich platelet-rich plasma obtained with the Mini GPS III Platelet Concentration System is an efficient source of paracrine molecules such as TGF-β1, EGF, PDGF-AA, PDGF-BB, IL-1β, IFN-α2, TNF-α, and IL-8 with the ability to concentrate those molecules above twice as baseline. The profile of growth factors and cytokines is different in PRP than in patients' own blood serum. For the above reason, PRP cannot simply be replaced by an increased serum volume, which may, however, be considered for selected cases as an alternative treatment option.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflict of interest with respect to the publication of this article.

Acknowledgments

This work was financially supported by the grant from the project entitled "Wroclaw Medical University as a Regional Center of Excellence in the field of medical sciences and health sciences" implemented under the funds of the Ministry of Science and Higher Education (Republic of Poland) in the program "Regional Initiative of Excellence" in the years 2019-2022, project number 016/RID/2018/19 (grant number RID.Z501.20.008).

Supplementary Materials

Supplementary Table 1: differences between males and females according to age, BMI, and biologically active compounds in whole blood, serum, and PRP. Values are presented as arithmetic mean (standard deviation) and median (Q1-Q3). The significance of the comparison is shown as p value. *(Supplementary Materials)*

References

[1] N. Hussain, H. Johal, and M. Bhandari, "An evidence-based evaluation on the use of platelet rich plasma in orthopedics - a review of the literature," *Sicot-J*, vol. 3, 2017.

[2] D. Man, H. Plosker, and J. E. Winland-Brown, "The use of autologous platelet-rich plasma (platelet gel) and autologous platelet-poor plasma (fibrin glue) in cosmetic surgery," *Plastic and Reconstructive Surgery*, vol. 107, no. 1, pp. 229–237, 2001.

[3] D. C. Tietze, K. Geissler, and J. Borchers, "The effects of platelet-rich plasma in the treatment of large-joint osteoarthritis: a systematic review," *The Physician and Sportsmedicine*, vol. 42, no. 2, pp. 27–37, 2014.

[4] G. Chellamuthu, S. Muthu, M. Khanna, and V. Khanna, "'Platelet-rich plasma holds promise in management of rheumatoid arthritis'—systematic review," *Rheumatology International*, vol. 41, no. 11, pp. 1895–1903, 2021.

[5] H. Badsha, G. Harifi, and W. D. Murrell, "Platelet rich plasma for treatment of rheumatoid arthritis: case series and review of literature," *Case Reports in Rheumatology*, vol. 2020, Article ID 8761485, 7 pages, 2020.

[6] S. Yan, B. Yang, C. Shang et al., "Platelet-rich plasma promotes the migration and invasion of synovial fibroblasts in patients with rheumatoid arthritis," *Molecular Medicine Reports*, vol. 14, no. 3, pp. 2269–2275, 2016.

[7] S. Tong, C. Zhang, and J. Liu, "Platelet-rich plasma exhibits beneficial effects for rheumatoid arthritis mice by suppressing inflammatory factors," *Molecular Medicine Reports*, vol. 16, no. 4, pp. 4082–4088, 2017.

[8] B. W. Oudelaar, J. C. Peerbooms, R. Huis In 't Veld, and A. J. Vochteloo, "Concentrations of blood components in commercial platelet-rich plasma separation systems: a review of the literature," *The American Journal of Sports Medicine*, vol. 47, no. 2, pp. 479–487, 2019.

[9] D. J. Sánchez-González, E. Méndez-Bolaina, and N. I. Trejo-Bahena, "Platelet-rich plasma peptides: key for regeneration," *International Journal of Peptide*, vol. 2012, pp. 1–10, 2012.

[10] S. Werner and R. Grose, "Regulation of wound healing by growth factors and cytokines," *Physiological Reviews*, vol. 83, no. 3, pp. 835–870, 2003.

[11] M. Dejnek, H. Moreira, S. Placzkowska et al., "Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: a preliminary study," *Advances in Clinical and Experimental Medicine*, vol. 30, no. 7, pp. 757–764, 2021.

[12] R. Evanson, M. K. Guyton, D. L. Oliver et al., "Gender and age differences in growth factor concentrations from platelet-rich plasma in adults," *Military Medicine*, vol. 179, no. 7, pp. 799–805, 2014.

[13] P. Jayaram, P. Yeh, S. J. Patel et al., "Effects of aspirin on growth factor release from freshly isolated leukocyte-rich platelet-rich plasma in healthy men: a prospective fixed-sequence controlled laboratory study," *The American Journal of Sports Medicine*, vol. 47, no. 5, pp. 1223–1229, 2019.

[14] G. Weibrich, W. K. G. Kleis, G. Hafner, and W. E. Hitzler, "Growth factor levels in platelet-rich plasma and correlations with donor age, sex, and platelet count," *Journal of Cranio-Maxillofacial Surgery*, vol. 30, no. 2, pp. 97–102, 2002.

[15] R. Zimmermann, D. Arnold, E. Strasser et al., "Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates," *Vox Sanguinis*, vol. 85, no. 4, pp. 283–289, 2003.

[16] D. M. Dohan Ehrenfest, L. Rasmusson, and T. Albrektsson, "Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF)," *Trends in Biotechnology*, vol. 27, no. 3, pp. 158–167, 2009.

[17] R. Dhurat and M. Sukesh, "Principles and methods of preparation of platelet-rich plasma: a review and author's perspective," *Journal of Cutaneous and Aesthetic Surgery*, vol. 7, no. 4, pp. 189–197, 2014.

[18] J. F. S. D. Lana, J. Purita, C. Paulus et al., "Contributions for classification of platelet rich plasma - proposal of a new classification: MARSPILL," *Regenerative Medicine*, vol. 12, no. 5, pp. 565–574, 2017.

[19] R. E. Marx, "Platelet-rich plasma (PRP): what is PRP and what is not PRP?," *Implant Dentistry*, vol. 10, no. 4, pp. 225–228, 2001.

[20] A. Mishra and T. Pavelko, "Treatment of chronic elbow tendinosis with buffered platelet-rich plasma," *The American Journal of Sports Medicine*, vol. 34, no. 11, pp. 1774–1778, 2006.

[21] L. Mazzucco, V. Balbo, E. Cattana, R. Guaschino, and P. Borzini, "Not every PRP-gel is born equal Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet®, RegenPRP-Kit®, Plateltex® and one manual procedure," *Vox Sanguinis*, vol. 97, no. 2, pp. 110–118, 2009.

[22] Y. Kobayashi, Y. Saita, H. Nishio et al., "Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations," *Journal of Orthopaedic Science*, vol. 21, no. 5, pp. 683–689, 2016.

[23] A. Cieślik-Bielecka, T. Bold, G. Ziółkowski, M. Pierchała, A. Królikowska, and P. Reichert, "Antibacterial activity of leukocyte- and platelet-rich plasma: an in vitro study," *BioMed Research International*, vol. 2018, Article ID 9471723, 8 pages, 2018.

[24] L. Zhang, S. Chen, P. Chang et al., "Harmful effects of leukocyte-rich platelet-rich plasma on rabbit tendon stem cells in vitro," *The American Journal of Sports Medicine*, vol. 44, no. 8, pp. 1941–1951, 2016.

[25] E. A. Sundman, B. J. Cole, and L. A. Fortier, "Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma," *The American Journal of Sports Medicine*, vol. 39, no. 10, pp. 2135–2140, 2011.

[26] J. H. Oh, W. O. O. Kim, K. U. Park, and Y. H. Roh, "Comparison of the cellular composition and cytokine-release kinetics of various platelet-rich plasma preparations," *The American Journal of Sports Medicine*, vol. 43, no. 12, pp. 3062–3070, 2015.

[27] J. Magalon, O. Bausset, N. Serratrice et al., "Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model," *Arthroscopy: The Journal of Arthroscopic & Related Surgery*, vol. 30, no. 5, pp. 629–638, 2014.

[28] T. N. Castillo, M. A. Pouliot, H. J. Kim, and J. L. Dragoo, "Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems," *The American Journal of Sports Medicine*, vol. 39, no. 2, pp. 266–271, 2011.

[29] S. A. Khan, J. Joyce, and T. Tsuda, "Quantification of active and total transforming growth factor-β levels in serum and solid organ tissues by bioassay," *BMC Research Notes*, vol. 5, no. 1, p. 1, 2012.

[30] T. Molloy, Y. Wang, and G. A. C. Murrell, "The roles of growth factors in tendon and ligament healing," *Sports Medicine*, vol. 33, no. 5, pp. 381–394, 2003.

[31] J. L. Dragoo, T. Korotkova, A. S. Wasterlain, M. A. Pouliot, H. J. Kim, and S. R. Golish, "Age-related changes of chondrogenic growth factors in platelet-rich plasma," *Operative Techniques in Orthopaedics*, vol. 22, no. 2, pp. 49–55, 2012.

[32] S. Lippross, B. Moeller, H. Haas et al., "Intraarticular injection of platelet-rich plasma reduces inflammation in a pig model of rheumatoid arthritis of the knee joint," *Arthritis and Rheumatism*, vol. 63, no. 11, pp. 3344–3353, 2011.

[33] W. Wang, J. Liu, B. Yang et al., "Modulation of platelet-derived microparticles to adhesion and motility of human rheumatoid arthritis fibroblast-like synoviocytes," *PLoS One*, vol. 12, no. 7, pp. 1–12, 2017.

[34] D. S. Saif, N. N. Hegazy, and E. S. Zahran, "Evaluating the efficacy of intra-articular injections of platelet rich plasma (PRP) in rheumatoid arthritis patients and its impact on inflammatory cytokines, disease activity and quality of life," *Current Rheumatology Reviews*, vol. 16, 2020.

Supplementary Table 1. Differences between males and females according to age, BMI and biologically active compounds in whole blood, serum and PRP. Values are presented as arithmetic mean (standard deviation) and median (Q1-Q3). The significance of the comparison is shown as *p*-value.

	Females		Males		
	Mean (SD)	**Median (Q1 – Q2)**	**Mean (SD)**	**Median (Q1 – Q2)**	**p**
Age [years]	49.69 (4.41)	50 (46.5 – 52)	48.47 (7.5)	50 (44 – 55)	0.582
BMI	26.1 (5.03)	25.08 (22.35 – 28.63)	28.68 (4.12)	27.34 (25.44 – 30.64)	0.130
PRP					
WBC [10^3/µl]	25.83 (7.15)	24.72 (21.05 – 31.36)	34.64 (9.96)	35.38 (24.7 – 45.23)	< 0.05
Neutrophiles [10^3/µl]	10.79 (5.77)	11.39 (6.83 – 13.66)	14.77 (7.11)	18.03 (10.2 – 20.25)	0.096
Lymphocytes [10^3/µl]	12.34 (4.19)	11.82 (9.73 – 13.72)	16.13 (5.27)	14.75 (12.03 – 21.08)	< 0.05
Monocytes [10^3/µl]	2.32 (0.71)	2.25 (1.96 – 2.89)	3.34 (1.16)	3.31 (2.71 – 4.37)	0.006
Eosinophiles [10^3/µl]	0.13 (0.16)	0.06 (0.03 – 0.18)	0.22 (0.16)	0.21 (0.05 – 0.31)	0.069
Basophiles [10^3/µl]	0.2 (0.15)	0.14 (0.1 – 0.31)	0.18 (0.09)	0.18 (0.11 – 0.26)	0.984
RBC [10^6/µl]	0.95 (0.52)	0.74 (0.62 – 1.02)	0.89 (0.46)	0.67 (0.52 – 1.23)	0.520
PLT [10^3/µl]	1108.5 (598.81)	1214.5 (515 – 1476)	1057.6 (369.26)	1032 (816 – 1296)	0.780
TGF-β1, free active [pg/ml]	392.45 (253.91)	336.5 (237.3 – 451.23)	373.6 (257.95)	343.26 (257.9 – 394.29)	0.890
EGF [pg/ml]	233.68 (134.68)	244.3 (101.51 – 312.78)	254.25 (177.58)	206.71 (116.43 – 404.62)	0.934
FGF-basic [pg/ml]	324.55 (159.75)	281.62 (225.27 – 397.97)	1198.18 (2922.48)	364.19 (299.8 – 428.55)	0.156
VEGF [pg/ml]	254.85 (389.29)	98.185 (38.54 – 287.08)	404.16 (399.48)	225.79 (83.68 – 683.32)	0.205
HGF [pg/ml]	228.21 (105.85)	213.93 (168.02 – 249.66)	234.46 (126.26)	203.45 (154.82 – 290.69)	0.983
PDGF-AA [pg/ml]	131264.78 (53824.16)	141055.15 (91260.69 – 181068.92)	134283.04 (55220.91)	132774.2 (90062.55 – 193193.8)	0.879
PDGF-BB [pg/ml]	47025.82 (17870.22)	50207.32 (31570.24 – 60480.01)	51402.72 (18623.90)	53419.26 (29826.86 – 62932.69)	0.510
IL-1β [pg/ml]	39.49 (21.41)	39.9 (19.83 – 49.41)	98.63 (71.71)	75.64 (34.73 – 151.71)	< 0.05
IFN-α2 [pg/ml]	27.78 (14.36)	22.59 (16.16 – 39.39)	50.96 (35.29)	47.88 (20.21 – 77.7)	< 0.05
IFN-γ [pg/ml]	5.65 (1.75)	4.87 (4.87 – 5.7)	7.81 (3.89)	6.39 (4.87 – 8.72)	0.101
TNF-α [pg/ml]	23.74 (13.65)	19.04 (13.02 – 31.76)	43.07 (34.15)	28.28 (13.54 – 69.04)	0.118
MCP-1 [pg/ml]	101.53 (57.44)	81.01 (65.57 – 136.93)	115.17 (76.32)	94.22 (63.97 – 151.18)	0.632
IL-6 [pg/ml]	15.81 (4.4)	14.49 (12.16 – 18.75)	21.99 (12.99)	16.01 (12.16 – 25.01)	0.271
IL-8 [pg/ml]	69.74 (52.34)	50.61 (43.55 – 82.66)	185.98 (163.13)	119.31 (51.99 – 316.42)	< 0.05
IL-10 [pg/ml]	14.7 (3.09)	14.11 (11.76 – 17.28)	23.56 (9.53)	24.34 (13.48 – 28.2)	< 0.05
IL-12p70 [pg/ml]	15.17 (4.69)	14.66 (10.66 – 18.2)	26.7 (13.29)	27.8 (13.93 – 34.35)	< 0.05
IL-17A [pg/ml]	2.45 (0.99)	1.94 (1.94 – 2.61)	3.96 (1.95)	3.93 (1.94 – 4.74)	< 0.05
IL-18 [pg/ml]	251.77 (141.57)	194.32 (155.87 – 334.12)	564.33 (327.8)	492.4 (296.32 – 959.07)	< 0.05
IL-33 [pg/ml]	128.97 (46.14)	112.15 (90 – 161.02)	227.37 (116.04)	224.97 (107.24 – 276.7)	< 0.05
Whole blood					
WBC [10^3/µl]	6.78 (1.06)	6.97 (5.72 – 7.78)	6.42 (1.6)	6.62 (4.61 – 7.74)	0.466

Neutrophiles [10^3/μl]	4.34 (1.09)	4.14 (3.21 – 5.3)	3.71 (1.29)	3.96 (2.44 – 4.94)	0.153
Lymphocytes [10^3/μl]	1.87 (0.51)	1.73 (1.64 – 1.98)	2.06 (0.52)	1.87 (1.55 – 2.59)	0.350
Monocytes [10^3/μl]	0.36 (0.09)	0.34 (0.29 – 0.44)	0.43 (0.11)	0.43 (0.35 – 0.51)	0.063
Eosinophiles [10^3/μl]	0.11 (0.06)	0.1 (0.07 – 0.13)	0.2 (0.14)	0.16 (0.09 – 0.24)	< 0.05
Basophiles [10^3/μl]	0.03 (0.02)	0.03 (0.02 – 0.04)	0.03 (0.01)	0.03 (0.02 – 0.04)	0.900
RBC [10^6/μl]	4.56 (0.37)	4.52 (4.38 – 4.81)	5.15 (0.28)	5.18 (4.89 – 5.42)	≤ 0.001
PLT [10^3/μl]	270.8 (66.39)	270 (238 – 312)	235.73 (47.27)	225 (196 – 292)	0.107
			Serum		
TGF-β1, free active [pg/ml]	144.37 (88.93)	130.21 (57.59 – 236.84)	159.5 (51.46)	167.12 (126.14 – 198.91)	0.583
EGF [pg/ml]	118.26 (52.13)	105.59 (72.08 – 159.2)	100.55 (34.34)	94.72 (78.05 – 130.18)	0.306
FGF-basic [pg/ml]	1065.19 (623.24)	773.2 (484.02 – 1720.4)	1125.0 (301.76)	1170.44 (967.45 – 1333.44)	0.570
VEGF [pg/ml]	141.68 (50.55)	154.04 (105.41 – 173.25)	165.8 (54.58)	151.81 (139.14 – 168.69)	0.612
HGF [pg/ml]	559.05 (260.44)	588.25 (321.61 – 739.51)	506.64 (137.52)	493.59 (394.27 – 557.95)	0.521
PDGF-AA [pg/ml]	25252.81 (13074.01)	23429.45 (18473.94 – 30788.92)	25687.25 (14918.73)	24049.49 (13143.73 – 32235.4)	0.934
PDGF-BB [pg/ml]	8840.36 (5556.49)	7291.22 (6157.65 – 13513.71)	9316.23 (7562.22)	6020.43 (4208.4 – 144.08.71)	0.848
IL-1β [pg/ml]	33.0 (46.04)	18.12 (18.12 – 18.12)	35.34 (52.13)	18.12 (18.12 -20.95)	0.357
IFN-α2 [pg/ml]	16.35 (0.75)	16.16 (16.16 – 16.16)	17.52 (3.62)	16.16 (16.16 – 16.16)	0.461
IFN-γ [pg/ml]	4.87 (1.1)	4.87 (4.87 – 4.87)	5.08 (0.79)	4.87 (4.87 – 4.87)	0.765
TNF-α [pg/ml]	16.99 (6.41)	13.02 (13.02 – 19.82)	20.25 (8.4)	18.59 (13.02 – 30.57)	0.434
MCP-1 [pg/ml]	169.66 (202.77)	75.31 (29.66 – 243.04)	106.25 (95.43)	82.92 (31.97 – 132.87)	0.854
IL-6 [pg/ml]	13.54 (3.42)	12.16 12.16 – 12.16)	12.67 (1.12)	12.16 (12.16 – 12.83)	0.782
IL-8 [pg/ml]	43.12 (84.09)	15.1 (12.14 – 35.97)	52.44 (112.2)	19.2 (12.14 – 30.94)	0.981
IL-10 [pg/ml]	12.53 (1.68)	11.76 (11.76 -12.42)	14.71 (5.42)	11.76 (11.76 – 15.19)	0.629
IL-12p70 [pg/ml]	13.43 (3.03)	12.23 (10.66 – 15.35)	14.38 (5.28)	10.66 (10.66 – 17.35)	0.777
IL-17A [pg/ml]	2.57 (0.83)	2.04 (1.94 – 3.01)	2.43 (0.94)	1.94 (1.94 – 2.43)	0.266
IL-18 [pg/ml]	200.66 (135.43)	165.39 (117.9 – 254.34)	315.77 (187.17)	281.27 (185.84 – 437.73)	0.060
IL-33 [pg/ml]	126.21 (51.97)	103.81 (83.24 – 160.89)	108.95 (39.59)	92.635 (83.24 – 111.78)	0.435

VI. PRACA NR 4:

Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma

Article

Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma

Maciej Dejnek [1,*], Helena Moreira [2], Sylwia Płaczkowska [3], Ewa Barg [2], Paweł Reichert [1] and Aleksandra Królikowska [4]

[1] Clinical Department of Trauma and Hand Surgery, Department of Trauma Surgery, Faculty of Medicine, Wroclaw Medical University, 50-556 Wroclaw, Poland; pawel.reichert@umw.edu.pl

[2] Department of Medical Science Foundation, Faculty of Pharmacy, Wroclaw Medical University, 50-556 Wroclaw, Poland; helena.moreira@umw.edu.pl (H.M.); ewa.barg@umw.edu.pl (E.B.)

[3] Teaching and Research Diagnostic Laboratory, Department of Laboratory Diagnostics, Faculty of Pharmacy, Wroclaw Medical University, 50-556 Wroclaw, Poland; sylwia.placzkowska@umw.edu.pl

[4] Ergonomics and Biomedical Monitoring Laboratory, Department of Physiotherapy, Faculty of Health Sciences, Wroclaw Medical University, 50-355 Wroclaw, Poland; aleksandra.krolikowska@umw.edu.pl

* Correspondence: maciej.dejnek@student.umw.edu.pl; Tel.: +48-717343800

Abstract: Autologous platelet-rich plasma (PRP) injection is an alternative treatment option for patients with lateral elbow tendinopathy. The treatment is supposed to accelerate tissue regeneration by providing high concentrations of growth factors derived from platelets. The aim of the study was to assess the relationship between the content of biologically active compounds in PRP and the clinical effect of the treatment. Thirty patients with lateral elbow tendinopathy treated with a single PRP injection, were evaluated. The pain intensity (measured by a visual analogue scale (VAS)), the pressure pain threshold (PPT), the grip strength and strength of the main arm and forearm muscle groups, and the functional outcome (measured by the Disability of Arm, Shoulder and Hand (DASH) and Patient-Rated Tennis Elbow Evaluation (PRTEE) questionnaires), were assessed before PRP injection and at one- and three-months follow-up. Flow cytometry measurements of the growth factors and inflammatory cytokines in PRP were performed, and the results were used to establish the relationship between those molecules and the clinical outcome. After three months from the intervention, the minimal clinically important difference in pain reduction and functional improvement was observed in 67% and 83% of patients, respectively. Positive correlations were found between the extent of pain reduction after three months and concentrations in the PRP of platelets, epidermal growth factor (EGF), vascular endothelial growth factor, and platelet-derived growth factors. The concentration of EGF in the PRP significantly correlated with an improvement in grip strength, strength of wrist extensors, and the size of functional improvement measured by the PRTEE. The local injection of PRP is a safe and effective treatment option for lateral elbow tendinopathy, and the clinical outcome is correlated with concentrations of its biologically active compounds.

Keywords: platelet-rich plasma (PRP); tennis elbow; lateral elbow enthesopathy; lateral epicondylitis; growth factors; cytokines

Citation: Dejnek, M.; Moreira, H.; Płaczkowska, S.; Barg, E.; Reichert, P.; Królikowska, A. Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma. *J. Clin. Med.* **2022**, *11*, 3687. https://doi.org/10.3390/jcm11133687

Academic Editor: Stasinopoulos Dimitrios

Received: 23 May 2022
Accepted: 23 June 2022
Published: 27 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Lateral elbow tendinopathy is a degenerative condition of proximal attachment of extensor muscles to the humeral epicondyle [1]. Among the best-described causes of the disease are repeated movements of the upper limb and lifting heavy objects [2]. Although the most commonly used name is 'tennis elbow', the disease is not always connected to sports activity [3]. It mainly occurs in manual workers, who perform multiple flexion and extension as well as torsional wrist movements and forceful griping at work [4]. The

disease affects 1–3% of the population, of both sexes, most often in the age between 40 and 60 years [5].

From the pathophysiological point of view, as a result of repeated microtraumas, abnormal angiofibroblastic remodeling develops on the site of muscle attachment, leading to increased pain sensation [6]. These changes result from incorrect repairing processes, consequentially interrupted by overload injuries [7] and extensor carpi radialis brevis (ECRB) and common extensor (CE) origins are mainly involved [6]. Conservative treatment is the method of choice at the beginning of the disease and avoiding limb overloading, changing harmful movement patterns at work, cooling, stretching and medications for pain control could be sufficient for the early stages [8]. A self-limiting character of the disease leads to spontaneous remission within a few weeks, and in most of the patients, the pain will resolve finally within a year [4]. Unfortunately, in some patients, severe pain intensity makes it impossible to perform simple everyday activities.

For patients in whom the first line treatment has failed, no sufficiently reliable treatment has been developed so far. The most commonly used second line treatment method is the local injection of corticosteroids [9]. Unfortunately, such treatment, although very effective in the short term, is associated with the highest rate of recurrence and numerous complications [10].

The treatment targeted at enhancing healing is the injection of autologous platelet-rich plasma (PRP) [11]. The procedure involves delivering to the site of injury, previously prepared patient's plasma with a higher than baseline platelet content. These platelets are supposed to locally release a large number of cytokines and growth factors, which should stimulate the natural repair processes [11]. Among the cytokines released from the platelets' α-granules, the best described are platelet-derived growth factors (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor-β1 [12]. Many other cytokines are also involved in healing processes and among the most important are Interferon- γ and -α (IFN-α, and IFN-γ), numerous interleukins (IL), metalloproteinases, and chemoattractant proteins [12]. Moreover, the influence of individual cytokines on the healing processes has been studied; however, evaluating their effects on living organisms is challenging due to their numerous mutual interactions, both stimulating and inhibiting.

There are many doubts about the efficacy of PRP injection in lateral elbow tendinopathy. Most publications have presented significant improvements, being better than corticosteroid injection at more than three months after treatment or even being equal to surgery [13–16]. On the other hand, some studies have reported similarly good outcomes after saline or autologous blood injection [14,17] while three months appeared to be the cut-off point for positive PRP treatment results. Conversely, patients, who are still suffering after an extended period, often do not want to wait any longer and start looking elsewhere for help [18]. An additional problem in assessing the effectiveness of treatment is the great variety of methods for obtaining PRP. Many commercial PRP kits differ in the way they separate the platelets from other cellular components, the amount of blood to be drawn from the patient, and the platelets' activation method [19]. This leads to a PRP with a different density of platelets or white blood cells and, consequently, a different density of growth factors and cytokines [20]. Previous studies have shown that both platelets and leukocytes in PRP can correlate with higher levels of growth factors; therefore, we decided to use a commercial kit that allows for obtaining both a high platelet and leukocyte content. Additional potential benefits of the use of a leukocyte-rich PRP include its antimicrobial properties and the ability to influence an immune response. The existing concerns about the negative impact on tissue healing due to an ability to stimulate catabolic reactions, and the release of pro-inflammatory cytokines and proteinases have not been confirmed in in vivo studies [15,16].

The aim of the study was to determine the relationship between the content of selected growth factors or inflammatory cytokines in PRP samples and the effectiveness of the PRP injection treatment in patients with lateral elbow tendinopathy. We hypothesized that

the greater the content of growth factors in the injected PRP, the greater the pain reduction, functional improvement, and improvement in muscle strength during three months of follow-up.

2. Materials and Methods

2.1. Ethical Standards

The study was carried out according to the Declaration of Helsinki, and was approved by the Bioethics Committee of Wroclaw Medical University (KB—26/2019, 21 January 2019). All patients agreed to participate in the study and signed an informed consent. The patients were assessed from February 2021 to May 2022 at the Department of Trauma Surgery.

2.2. Study Design

The study was designed as a part of a single-center, double-blinded, prospective randomized controlled trial titled 'Evaluation of Clinical Efficacy and Comparison of Autologous Platelet Rich Plasma, Hyaluronic Acid, Corticosteroid and Saline Injections for Treating Lateral Humeral Epicondylopathy' registered in clinicaltrials.gov under the identifier, NCT04521387. The study was double-blinded, so that the patient and the assessor evaluating the outcomes had no information about the type of treatment. The patients were not informed of the kind of injection they received, and the syringe was covered with opaque tape to mask its contents during administration. Each patient had a blood sample taken for complete blood counts, so that they could not easily guess if they were assigned to the PRP group, which was the only group that required blood donation. The only person who knew the allocation of patients to the groups was the primary supervisor, who decided to unblind the results of the three-months follow-up of patients in the PRP group to present the correlation results between the growth factors and cytokines in the current publication. The results of all group comparisons will be presented at the end of the one-year observation period in accordance with the original protocol, which is expected to be in the year 2023.

2.3. Population

We enrolled patients with pain on the lateral side of the elbow joint after the failure of first line treatment for at least three months, with confirmation of lateral elbow enthesopathy in at least one provocative test (the Cozen's test, Thomson's test, Maudsley's test, or Chair test) [1]. Patients with previous invasive treatment on the lateral aspect of the involved elbow (including previous injections), with hematological diseases, diabetes, gout, malignant tumors, advanced osteoarthritis of the elbow joint, nervous system diseases related to the upper limb, suspicion of an infectious process, who were pregnant, or taking medications that may affected platelet function or the coagulation system were excluded from the study. The patients learned about the possibility of taking part in the study from the University's website and social media and during a visit at an outpatient clinic. If they volunteered to participate in the study, they were recruited after meeting the inclusion criteria at the initial visit. A bilateral X-ray of the elbow and ultrasound examination were performed to exclude other pathologies and to confirm changes typical for lateral elbow enthesopathy (see Figure 1) [21]. Information about the patients' age, height, and weight (for body mass index, BMI calculation), type of work, duration of symptoms, smoking status, sports activity, medications, any other diseases, and previous treatments were collected during a medical interview.

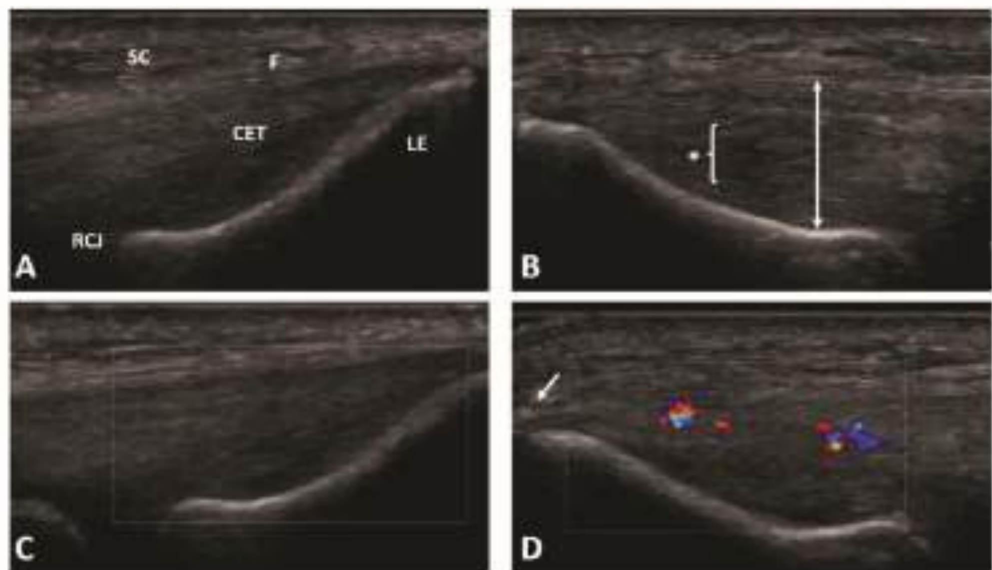


Figure 1. Ultrasound examination of the patient's both elbows for lateral elbow tendinopathy confirmation: (**A**) healthy lateral epicondyle region of the contralateral elbow; (**B**) pathological common extensor tendon thickening and region with diffuse hypoechogenicity typical for lateral elbow tendinopathy; (**C**) healthy common extensor tendon without activity in color Doppler; (**D**) neoangiogenesis shown as increased activity in color Doppler in the tendinopathic region. SC—subcutaneous tissue; F—fascia; CET—common extensor tendon; LE—lateral epicondyle; RCJ—radiocapitellar joint; * and bracket—diffuse hypoechogenic region; two-head arrow—tendon thickness measurement; arrow—enthesophyte.

2.4. Intervention

Under aseptic conditions, from each patient, 27 mL of blood was drawn from a cubital vein of the opposite elbow into a 30 mL syringe filled with 3 mL of anticoagulant citrate dextrose solution A (ACD-A). An additional two ml was drawn to the probe with ethylenediaminetetraacetic acid (EDTA) and was used for the complete blood count analysis using the Mindray BC-5150 automatic laboratory analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Shenzhen, China).

A 30 mL measure of blood mixed with anticoagulant was transferred to a Mini GPS III Platelet Concentration System (Biomet Inc., Warsaw, IN, USA) and then centrifugated for 15 min with 3200 revolutions per minute (RPM), at a force of 1740× *g*. According to the manufacturer's protocol, about 3 mL of liquid-form leukocyte-rich platelet-rich plasma (LR-PRP) was obtained. Platelet-poor plasma was removed from the separation tube, and the RBC layer was left in the tube under the separation membrane. For better visualization, the step-by-step process of obtaining PRP by the Mini GPS III is illustrated in Figure 2. One ml of PRP was reinjected into an Eppendorf polypropylene tube for further cell content analysis, and then the samples were activated by a double freeze–thaw process for 30 min in each step according to the method proposed by Zimmermann et al. [22]. This is a relatively simple and convenient method of platelet activation, especially in the planned storage of a frozen preparation. It allows the release of growth factors in a similar way to other activation methods, including in vivo activation by contact with native collagen. In the next step, the samples were frozen and stored at −80 °C until the time of the final growth factors and inflammatory cytokines analysis. The time between the blood draw, PRP separation, injection, complete blood count analysis, activation, and freezing for further storage did not exceed 1 h. The whole process was conducted in daylight at room temperature.

Five minutes before the injection of PRP, 1–2 mL of local anesthetic (1% lidocaine) was injected subcutaneously into the epicondyle region. Then, under aseptic conditions, under the guidance of ultrasonography, 2 mL of non-activated, liquid LR-PRP was injected into the ECRB and CE attachment to the lateral humeral epicondyle using the peppering technique

from one access through the skin and with multiple punctures (approximately 10 times) through the fascia to the periosteum. The PRP was injected above and under the fascia and into the tendon lesions identified with the ultrasonography. The 21G needle was used for that purpose. A sterile dressing was applied (see Figure 3). After injection, the patients were observed for approximately 30 min. Patients were instructed to avoid overloading activities and to start stretching exercises after a week from the injection. The inclusion of eccentric exercises to strengthen the extensors of the forearm was recommended in the case of a pain reduction below three on the visual analogue scale (VAS), not earlier than two weeks after the intervention.

Figure 2. Step-by-step preparation process for obtaining LR-PRP by the Mini GPS III Platelet Concentration System (Biomet Inc., Warsaw, IN, USA): (**A**) empty separation tube; (**B**) addition of 30 mL whole blood mixed with anticoagulant; (**C**) separation tube filled with patient's blood; (**D**) single centrifugation for 15 min; (**E**) the Mini GPS III tube after centrifugation with blood separated into three layers: *I*—platelet-poor plasma, *II*—platelet-rich plasma and buffy coat, *III*—red blood cells; (**F**) platelet-poor plasma removal; (**G**) platelet-rich plasma taken out of the tube into a syringe.

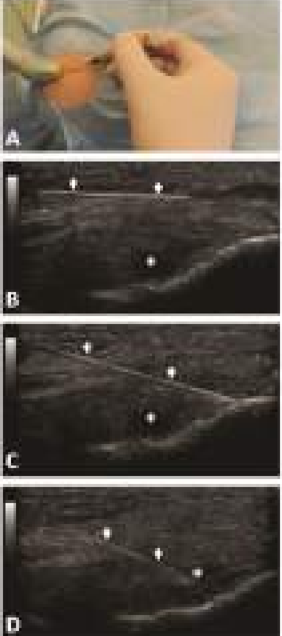


Figure 3. Presentation of ultrasound-guided PRP injection technique into common extensor tendon: (**A**) point of skin puncture with a needle guided by ultrasound probe in sterile conditions; (**B**) injection of 1–2 mL of 1% lidocaine in the subcutaneous region; (**C**) the peppering technique with multiple needle punctures through the fascia to the periosteum; (**D**) PRP injection into the hypoechogenic region of ECRB. PRP—platelet-rich plasma; ECRB—extensor carpi radialis brevis; arrows—view of the needle position under ultrasound; *—hypoechogenic region of the tendon.

2.5. Evaluation of Biologically Active Compounds

The two LEGENDplex multiplex immunoassays based on fluorescence-encoded beads and flow cytometric measurements were used to assess the content of cytokines and growth factors in the PRP. The first, LEGENDplex TM Custom Human 7-plex Panel (BioLegend, San Diego, CA, USA), is a custom-made panel dedicated to our study to estimate the concentration of the most important growth factors derived by platelets: transforming growth factor-β1 (TGF-β1, free active), epidermal growth factor (EGF), fibroblast growth factor-basic (FGF-basic), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), platelet-derived growth factor-AA (PDGF-AA), and platelet-derived growth factor-BB (PDGF-BB). The second, LEGENDplex TM Human Inflammation Panel 1 (BioLegend, San Diego, CA, USA), is included in the manufacturer's standard offer and was used to evaluate the inflammatory cytokine content in the samples tested: Interleukin-1β (IL-1β), Interferon-α2 (IFN-α2), Interferon-γ (IFN-γ), Tumor Necrosis Factor α (TNF-α), Monocyte Chemoattractant Protein-1 (MCP-1), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-10 (IL-10), Interleukin-12p70 (IL-12p70), Interleukin-17A (IL-17A), Interleukin-18 (IL-18), Interleukin-23 (IL-23), and Interleukin-33 (IL-33).

At the time of further analysis, all samples were thawed to room temperature and centrifuged for 5 min at 2500 RPM (350× *g*) in a Micro Star 17 microcentrifuge (VWR International Company, Thermo Electron LED, Germany) and diluted 2× in the assay buffer. Then, the growth factors and cytokine content analysis was performed according to the manufacturer's procedure for the LEGENDplex using the CyFlow Cube8 flow cytometer (Sysmex-Partec, Görlitz, Germany), applying a 488 nm laser with a 536/40 (BP) filter for the PE fluorochrome, and a 638 nm laser with 675/20 (BP) for the APC fluorochrome. The LEGENDplex TM Data Analysis Software version 8.0 (Vigene Tech Inc., Carlisle, MA, USA) was used for the analysis of the results. The concentration of each growth factor/cytokine was determined by means of a standard curve generated during the performance of the assay.

2.6. Evaluation of Treatment Efficacy

Just before the injection and consecutively one and three months later, the patients were evaluated according to pain intensity, muscle strength, and everyday functioning. The pain intensity was assessed with the VAS, which is a visual representation of a numerical scale from 0 (no pain) to 10 (the worst pain imaginable). After a week from the injection, we asked by phone about the patients' average pain intensity. Patients reported their average pain intensity in the VAS, of the current day and during the provocation tests. We used the five most commonly used provocation tests for tennis elbow: Cozen's test, Mill's test, Maudsley's lateral epicondylitis test, Thomson's test, and the Chair test [1]. The tests were interpreted as positive if the pain in the area of the lateral epicondyle increased during specific tasks. For the Cozen's test, this is the extension of the wrist against resistance with the forearm pronated, and the elbow flexed to 90 degrees. In the Thomson's test, the patient performs the same movement with the extended elbow. The starting position in the next two tests is the same as in the Cozen's test. In the Mill's test, the patient is trying to supinate the forearm against resistance and in the Maudsley's test, the patient is trying to fully extend the third finger against resistance. In addition, the Maudsley's test modification with an extended elbow was performed. The last-mentioned provocation test is the so-called Chair test, when the patient tries to lift the chair by holding the backrest with the grip, and having the elbow in extension. All these tests require contraction of the extensor muscles in the forearm, causing pain in the affected proximal attachment (see Figure 4).

Figure 4. Illustration of the assessment of provocation tests and the pressure pain threshold measurement: (**A**) Cozen's test; (**B**) Mill's test; (**C**) Maudsley's test; (**D**) Thomson's test; (**E**) Chair test; (**F**) pressure pain threshold measurement.

The pressure pain threshold (PPT) was assessed using the Wagner FPIX 25 Pain Test Algometer (Wagner Instruments, Riverside, CT, USA). This is a digital algometer with a hard rubber tip of 1 cm^2 surface. The algometer was applied to the site of greatest tenderness in the epicondyle area and pressed at a rate of 1 newton (N) per second. The measurement was read at the moment when the sensation of touch changed into the weakest pain sensation declared by the patient. This means that the lower the measurement reading (i.e., less pressure force needed to induce pain), the greater the tenderness. To assess the patient's pain sensitivity, the test was also performed on the same region of the opposite limb. The test was then repeated, and the final score was calculated as the mean of two consecutive measurements for each elbow. In all cases the PTT assessment was carried out by the same rater.

Consecutively, the strength of the following muscle groups was evaluated using a microFET2 (Hoggan Scientific, Salt Lake City, UT, USA) dynamometer: elbow extensors and flexors, forearm supinators and pronators, and wrist extensors. The measurements were performed in a lying position. The tests were performed in accordance with the manufacturer's recommendations. All the strength measurements were carried out by the same rater.

The grip strength assessment was carried out using the BIMS Digital Grip Dynamometer (Baseline, Washington, DC, USA). It was measured twice with a 1-min-long interval. The patient was comfortably placed with the elbow flexed to 90 degrees and the forearm in a neutral position resting on the armchair and asked to squeeze the dynamometer as hard as possible [23]. The patients were instructed to stop the test in the case of worsening pain symptoms, which was recorded.

The functional outcomes were measured using polish versions of two functional questionnaires: the Disability of Arm, Shoulder, and Hand Questionnaire (DASH), which is most commonly used for upper limb function evaluation, and the Patient-Rated Tennis Elbow Evaluation (PRTEE), which is best validated for the lateral elbow enthesopathy [24–27]. The DASH questionnaire consists of 30 questions about daily functioning. The patient answers each question on a 5-point scale, where 1 is the best and 5 is the worst outcome. The obtained sum is converted to a 100-point scale, taking into account the questions that the patient did not answer. The PRTEE questionnaire has two sections. The first one assessing pain intensity contains 5 questions rated from 0 (no pain) to 10 (worst imaginable pain). The second part assesses the function of the limb and includes 10 tasks rated from 0 (no difficulty) to 10 (unable to do). The final result (0–100) is the sum of the pain scale score and half of the functional scale score. In both questionnaires, the higher the score, the worse the function.

In addition, the patients were asked to assess both elbows with the Subjected Elbow Value (SEV), which was proven to be an easily administered, responsive, valid tool to assess

the condition of the elbow [28]. The SEV is defined as the patient's subjective estimation of the elbow as a percentage of a normal elbow, which would correspond to 100%.

The treatment was considered effective if a minimal clinically important difference (MCID) occurred in pain reduction, or a functional improvement between the baseline and follow-up periods. According to the literature, a MCID is equal to 1.5 points for the VAS, 15.8 points for the DASH, and 11 points for the PRTEE [16].

2.7. Statistical Analysis

The Shapiro–Wilk test was performed to assess the normal distribution of the results obtained. Data with a normal distribution were presented as an arithmetic mean and standard deviation (SD). Data with a non-normal distribution were described using the median and quartile distributions (Q1–Q3). The size of a treatment efficacy was presented as the change (Δ) between the baseline results and those at the 1st and 3rd month (Δ1 and Δ3, respectively). The significance of the differences between the subsequent follow-up periods was tested with the Student's *t*-test for paired samples with a normal distribution and the Wilcoxon signed-rank test for nonparametric comparison of two matched samples. To assess the potential relationship between the biologically active compounds in the PRP and the efficacy of treatment, the Pearson's correlation coefficient was established. The magnitudes of all the bivariate associations were classified as negligible (0.00–0.30), low (0.31–0.50), moderate (0.51–0.70), high (0.71–0.90), and very high (0.91–1.00). To find a significant moderate correlation ($r \geq 0.50$) with a satisfying test power ($1\text{-}\beta > 0.8$), the required sample size was estimated at 27. For the statistical calculations, the computer software Statistica 13.3 software (TIBCO Software Inc., Pittsburgh, PA, USA) was used. The significance of the results was established at $p < 0.05$.

3. Results

3.1. Pre-Treatment Evaluation

The patients were enrolled in the study in the years 2021–2022. Their main characteristics and baseline values are presented in Table 1. All 30 patients appeared at the one and three months post-intervention follow-up visits. Among unrelated conditions, they had hypertension ($n = 4$), lumbar discopathy ($n = 4$) and depression ($n = 2$). All patients underwent ineffective first-line conservative treatment, including stretching and strengthening exercises ($n = 21$), using an orthotic device for tennis elbow ($n = 5$), manual therapy ($n = 10$), laser therapy ($n = 4$), shockwave ($n = 2$), iontophoresis ($n = 3$), and cryotherapy ($n = 3$).

Table 1. The main baseline characteristics and the values of the pretreatment clinical evaluation.

Patients' Characteristics		**Baseline Evaluation Values**	
Age *	49.0 (6.12)	VAS 0–10 *	5.07 (1.76)
Sex (*n* (%))		*Cozen's test*	5.42 (2.55)
Female	15 (50)	*Mill's test*	4.25 (3.09)
Male	15 (50)	*Maudsley's test*	2.67 (2.54)
BMI *	27.5 (4.67)	*Maudsley's test with extended elbow*	6.42 (2.24)
Hand dominance (*n* (%))		*Thomson's test*	7.50 (2.18)
Right	27 (90)	*Chair test*	6.00 (2.24)
Left	3 (10)	PPT in N *	25.38 (11.76)
Affected elbow (*n* (%))		Pain sensitivity in N *	42.99 (16.99)
Right	19 (63)	Strength of muscle groups *	
Left	11 (37)	*Grip* in MAX kg	32.45 (14.28)
Dominant hand affected (*n* (%))	22 (73)	*Elbow flexion in N*	234.97 (90.34)
Physical labor (*n* (%))	15 (50)	*Elbow extension in N*	175.96 (66.71)
Regular sports activity (*n* (%))	14 (47)	*Wrist flexion in N*	159.03 (61.28)

Table 1. *Cont.*

Patients' Characteristics		Baseline Evaluation Values	
Current smokers (*n* (%))	3 (10)	*Wrist extension in N*	116.44 (52.47)
Alcohol consumption (*n* (%))		*Forearm supination in N*	26.05 (12.13)
occasionally (≤1 dose per week)	22 (73)	*Forearm pronation in N*	36.64 (18.35)
not at all	8 (27)		
Duration of symptoms in months		DASH *	37.28 (17.17)
(mean (SD))	17.33 (25.38)	PRTEE *	45.98 (19.17)
(median (MIN–MAX))	5 (3–120)	SEV *	47.50 (17.36)

* The values expressed as arithmetic mean (standard deviation). BMI: body mass index; VAS: visual analogue scale; PPT: pressure pain threshold; DASH: Disability of Arm, Shoulder and Hand questionnaire; PRTEE: Patient-Rated Tennis Elbow Evaluation; SEV: subjected elbow value.

The comparison between the complete blood count in whole blood and in PRP samples is presented in Table 2. The platelet concentration in PRP increased 4.38 (SD = 1.67) times than in the baseline. The concentration of WBC increased 4.60 times (SD = 1.12) than in the baseline. The RBC concentration decreased 0.21 (SD = 0.16) times than in the baseline. The mean platelet volume (MPV) in PRP was 9.67 fL (SD = 0.85). The growth factor and inflammatory cytokine concentrations in the PRP are presented in Supplementary Table S1.

Table 2. Differences in cellular content between whole blood and PRP. Values are presented as arithmetic mean (standard deviation).

	Whole Blood	PRP	*p*
PLT [$10^3/\mu L$]	252.72 (60.34)	1073.63 (498.63)	≤0.001
WBC [$10^3/\mu L$]	6.57 (1.36)	30.01 (9.74)	≤0.001
Neutrophiles	4.05 (1.23)	12.70 (6.77)	≤0.001
Lymphocytes	1.95 (0.52)	14.12 (5.12)	≤0.001
Monocytes	0.39 (0.1)	2.80 (1.09)	≤0.001
Eosinophiles	0.15 (0.12)	0.17 (0.17)	0.64
Basophiles	0.03 (0.02)	0.20 (0.12)	≤0.001
RBC [$10^6/\mu L$]	4.89 (0.41)	1.03 (0.77)	≤0.001

PRP: platelet-rich plasma; PLT: platelets; WBC: white blood cells; RBC: red blood cells. The significance of the Student's *t*-test for paired samples comparison is shown as the *p*-value.

3.2. Treatment Efficacy and Complications

No infections, neural lesions, collateral ligaments disruptions or joint cartilage damage were found during the three-month period. The main side effect was the intensification of pain, which was observed in five patients after a week, in four patients after a month, and in two patients after three months. Among these last two patients, one decided to undergo surgery, and the other decided to change their employment, in which lifting heavy objects was not required. The treatment was considered successful for pain reduction (MCID 1.5 points in the VAS) after one month in 15 patients, which increased to 20 patients after three months. After three months, pain completely disappeared only in five patients and did not change at all in two patients. A successful functional improvement measured by a difference in the DASH (MCID 15.8 points) after one and three months from injection, was established for 11 and 20 patients, respectively. A successful functional improvement measured by a difference in the PRTEE (MCID 11 points) after one and three months from injection, was established for 18 and 25 patients, respectively.

There was a significant decrease in pain intensity measured with the VAS at all follow-up points and both the mean values for the current day and during the provocation tests had improved. The decrease between the baseline pain and consecutive periods was: after one week 0.82 (SD = 2.10, $p < 0.05$), after one month 1.87 (SD = 2.3, $p \leq 0.001$), and after three months 2.70 (SD = 2.73, $p \leq 0.001$), see Figure 3. The values for each follow-up are presented in Supplementary Table S2.

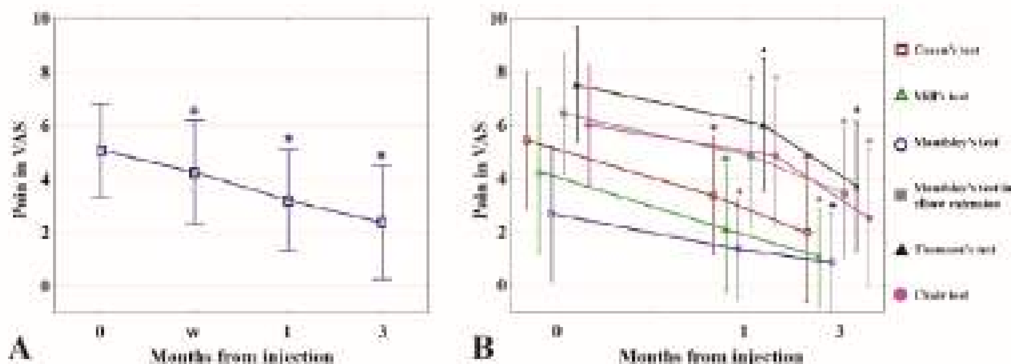


Figure 5. Change in pain intensity during follow-up measured in visual analog scale (VAS): (**A**) mean and SD pain intensity for a current day; (**B**) mean and SD pain intensity during provocation tests. *w*: one week; $^{+}$ $p < 0.05$ (compared to baseline); * $p \leq 0.001$ (compared to baseline).

The pressure pain threshold assessment showed a decrease in local tenderness by increasing the force needed to induce pain by 2.68 (SD = 9.27, $p = 0.102$) and 5.06 (SD = 11.98, $p < 0.05$), after one and three months, respectively. The values are presented in Figure 6.

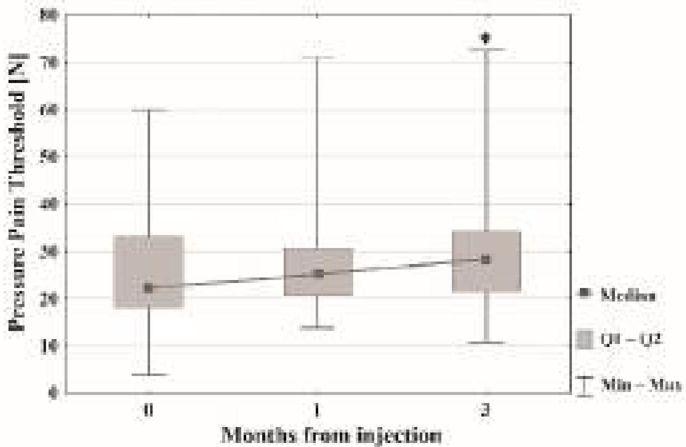


Figure 6. Result of pressure pain threshold assessment before, 1 and 3 months after intervention. * $p < 0.05$ (PPT 0 vs. PPT 3).

Three months after injection, most of the selected muscle groups improved in strength but a significant difference was only observed among the grip, elbow flexion, wrist extension, and forearm pronation strength (see Table 3).

Table 3. The strength of individual muscle groups before treatment and during follow-up visits.

	Baseline	after 1 Month	p^1	after 3 Months	p^2
Grip (MAX kg)	32.45 (14.28)	33.43 (13.63)	0.73	35.96 (14.46)	<0.05
Elbow flexion (N)	234.97 (90.34)	236.83 (91.14)	0.81	246.44 (84.57)	<0.05
Elbow extension (N)	175.96 (66.71)	172.07 (54.51)	0.43	172.17 (56.83)	0.39
Wrist flexion (N)	159.03 (61.28)	151.81 (48.27)	0.30	162.07 (59.05)	0.40
Wrist extension (N)	116.44 (52.47)	121.50 (51.14)	0.80	140.46 (49.45)	<0.05
Forearm supination (N)	26.05 (12.13)	24.71 (10.54)	0.31	29.05 (15.74)	0.07
Forearm pronation (N)	36.64 (18.35)	40.24 (17.93)	0.09	46.44 (20.15)	≤0.001

Values are presented as arithmetic mean (standard deviation). *p*-value represents significance of comparison between time periods: p^1 – baseline vs. 1st month; p^2 – baseline vs. 3rd month.

Scores from both the functional questionnaires decreased significantly during the follow-up, which indicates a functional improvement. The DASH score decreased by

12.50 (SD = 10.71, $p \leq 0.001$) and 22.11 points (SD = 16.52, $p \leq 0.001$) after one and three months, respectively. The PRTEE score decreased by 17.25 (SD = 15.06, $p \leq 0.001$) and 27.28 (SD = 21.28, $p \leq 0.001$) points after one and three months, respectively. The SEV showed a significant increase from 47.50% (SD = 17.36) before treatment to 67.17% (SD = 14.84, $p \leq 0.001$) after one month, and 73.77% (SD = 21.04, $p \leq 0.001$) at the final follow-up. A comparison between the different time points is presented in Figure 7.

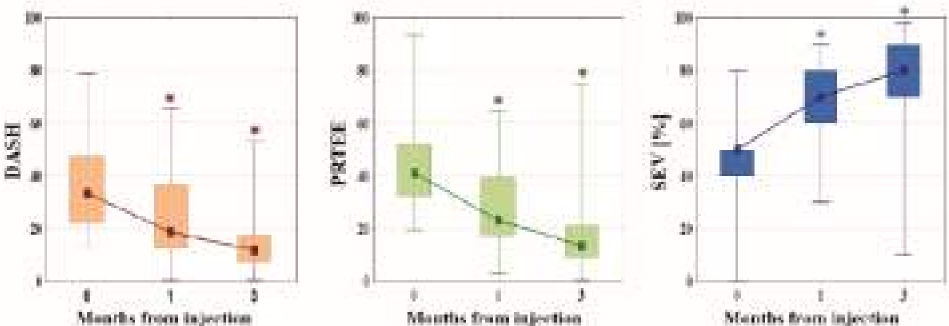


Figure 7. Results of DASH, PRTEE, and SEV scores before, 1, and 3 months after intervention. Square: median; box: Q1–Q2; whisker: MIN–MAX; * $p \leq 0.001$ (comparison with baseline value); DASH: Disability of Arm, Shoulder and Hand questionnaire; PRTEE: Patient-rated tennis elbow evaluation; SEV: subjected elbow value.

3.3. Correlation between Biologically Active Compounds in PRP and Treatment Efficacy

A significant moderate positive Pearson's correlation was found between the PLT concentration in the PRP and a decrease in pain intensity at the 3rd month after injection ($r = 0.56$, $p \leq 0.001$). There was also a positive low correlation between the PLT in the PRP and a PPT improvement ($r = 0.46$, $p < 0.05$). No correlation was found between the WBC or RBC content in the PRP and pain improvement at any point in the follow-up.

Between the growth factors and a pain decrease, only several significant correlations were found, but most of them were low. The concentration of EGF in the PRP had a positive moderate Pearson's correlation with a VAS improvement three months after injection ($r = 0.51$, $p < 0.05$). Significant low positive correlations were found between pain decrease after three months and the VEGF ($r = 0.36$, $p < 0.05$), PDGF-AA ($r = 0.37$, $p < 0.05$) and PDGF-BB ($r = 0.44$, $p < 0.05$). Additionally, a PPT improvement significantly correlated at a low level with the PDGF-BB concentration in the PRP after three months ($r = 0.38$, $p < 0.05$).

No significant correlation was found between the content of inflammatory cytokines and pain improvement. Selected significant positive correlations between the size of the decrease in pain intensity and biologically active compounds are presented in Figure 8.

Among the muscle groups whose strength improved significantly after three months from the injection (grip, elbow flexion, wrist extension, and forearm pronation), no correlation was found with the PLT, WBC, or RBC in the PRP. A significant positive low correlation was found between the EGF and a change in grip strength ($r = 0.44$, $p < 0.05$), and also in wrist extension ($r = 0.41$, $p < 0.05$). The MCP-1 content in the PRP significantly negatively correlated with the improvement in strength during elbow flexion ($r = -0.36$, $p < 0.05$).

A significant moderate positive correlation was found between the PLT content in the PRP and an SEV improvement at the 1st ($r = 0.54$, $p < 0.05$) and 3rd ($r = 0.53$, $p < 0.05$) month after injection. A significant positive low correlation was found between IL-18 in the PRP and a change in SEV after one month ($r = 0.37$, $p < 0.05$) but not after three months. No correlation was found between the cell content in the PRP and changes in the functional questionnaires score. A significant but low positive correlation was found between the EGF concentration in the PRP and a decrease in the PRTEE (functional improvement) after three months ($r = 0.37$, $p < 0.05$). Comparing the inflammatory cytokines content in the PRP and changes in the functional questionnaires, we found low negative correlations between

IL-10 and a PRTEE decrease after one and three months ($r = -0.37$, $r = -0.37$, and $p < 0.05$, respectively), between IL-33 and a PRTEE decrease after one and three months ($r = -0.38$, $r = 0.44$, and $p < 0.05$, respectively), and between IL-17A and a PRTEE decrease after three months ($r = -0.37$, $p < 0.05$). A decrease in the DASH score after three months negatively correlated with the IL-33 concentration in the PRP ($r = -0.39$, $p < 0.05$).

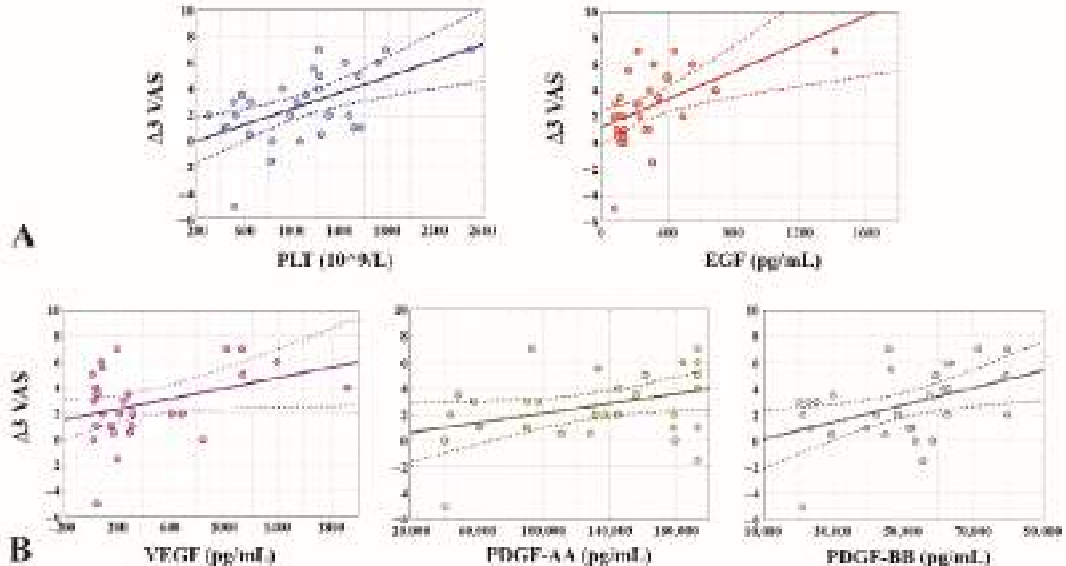


Figure 8. Significant positive Pearson's correlations between biologically active compounds in PRP and size of reduction in pain intensity after 3 months from intervention (Δ3 VAS = VAS 0 − VAS 3). (**A**) Significant moderate correlations ($r = 0.56$, $r = 0.51$, respectively). (**B**) Significant low correlations ($r = 0.36$, $r = 0.37$ and $r = 0.44$, respectively). PRP: platelet-rich plasma; PLT: platelets; EGF: epidermal growth factor; VEGF: vascular endothelial growth factor; PDGF-AA: platelet-derived growth factor-AA; PDGF-BB: platelet-derived growth factor-BB.

3.4. Correlation between Treatment Efficacy and Baseline Characteristics

No correlations were found between a VAS improvement during the follow-up and age, BMI, pain sensitivity, or duration of symptoms. A significant moderate positive correlation was found between the baseline VAS and size of the VAS reduction after one and three months ($r = 0.59$, $p < 0.05$ and $r = 0.63$, $p \leq 0.001$, respectively). A significant negative correlation was also found between the baseline SEV and size of the improvement in SEV after one and three months ($r = -0.74$, $p < 0.05$ and $r = -0.59$, $p \leq 0.001$, respectively), a decrease in the VAS after three months ($r = -0.54$, $p < 0.05$), a decrease in the DASH after three months ($r = -0.44$, $p < 0.05$), or a decrease in the PRTEE after three months ($r = -0.43$, $p < 0.05$). Additionally, high baseline values of the functional questionnaires were significantly correlated with the size of reduction after three months ($r = 0.73$, $p \leq 0.001$ for the DASH and $r = 0.69$, $p \leq 0.001$ for the PRTEE). The above correlations suggest that the worse the symptoms are before treatment, the greater the improvement that could be expected.

4. Discussion

Due to the aim of the study, positive correlations were found between the size of pain reduction after three months from the intervention, measured by a VAS and several growth factor concentrations in PRP: EGF, VEGF, PDGF-AA and PDGF-BB. A significant positive correlation was also found between the size of the pain reduction and the concentration of PLT in the PRP. After three months, the EGF concentration in the PRP significantly correlated with an improvement in grip strength, wrist extension strength, and functional improvement size measured by the PRTEE. Some negative correlations were found between

several inflammatory cytokines (IL-10, IL-33, and IL-17A) and changes in the functional questionnaires in the 3rd month after the intervention.

In our study, most patients were treated successfully after three months. Twenty patients (67%) reached a minimal clinically important difference in pain measured by the VAS, and in functional improvement measured by DASH. Even more patients (83%) reached a MCID in functional improvement measured by the PRTEE. Additionally, we established that the worse the pain, SEV or functional score had been at the beginning, the better the improvement that was obtained after three months.

Problems in assessing the effectiveness of PRP treatments start with the definition. Some researchers declare that PRP should be plasma with a platelet density above 1 million per µL [29]. In their review, Oudelaar et al., found that four among seven investigated commercially available systems were able to meet this criterion [19]. Although the mean PLT concentration in the PRP in our study was more than 1 million per µL (1,073,630 platelets per µL), only 17 out of the 30 samples met this criterion (range 289,000–2,491,000 platelets per µL). On the other hand, there are also researchers who believe that a platelet level > 200,000 per µL is sufficient to obtain a clinical effect [30]. All our PRP samples reached the above value. Additionally, significant differences in the content of WBC could lead to a product with completely different biological properties [19,31,32]. This differentiation made it necessary to propose a precise classification that would allow a reliable assessment of the PRP used. One of the first widespread classifications divided the PRP according to the leukocyte and fibrin content [33]. As this does not solve all the problems, subsequent classifications have proposed a division according to the leukocyte content, high ($\geq$5 times the baseline) or low platelet concentration, and the presence of additional PRP activation [34]. Further classifications added new parameters that seemed to be significant, such as the description of the centrifugation process, the separation technique, the injection technique, the presence of light activation, and the presence of RBC in the final product [35]. A consensus has even been developed on what information should be described in a study using PRP [36]. The separation system used by us is one of the most frequently used, as it enables to obtain PRP with a high platelet density (4–6 folds than the baseline) in a reproducible manner [20]. According to the MARSPIL classification, in our study we used leukocyte-rich, red blood cells-rich, non-activated, 4–6 times greater than the baseline platelet-rich plasma, handmade with a single-spin centrifugation and injected with ultrasound guidance; what should be reported as $M_{(H)}$, $A_{(A-)}$, $R_{(RBC\text{-}R)}$, $S_{(Sp1)}$, $P_{(PL\ [4\text{-}6])}$, $I_{(G+)}$, $L_{(Lc\text{-}R[4\text{-}6])}$, $L_{(A-)}$ [35]. The individual capital letters of the abbreviation in the above classification refers to: the preparation method (M), presence of activation (A), presence of red blood cells (R), number of spins (S), platelet concentration comparing to the baseline (P), presence of imaging guidance during injection (I), presence and concentration of leukocytes comparing to the baseline (L), and the presence of light activation (L), respectively. Each of the above should be clarified by adding the individual abbreviations below: whether it was prepared by an automated manner (machine: M) or handmade (H); the spin number (Sp1 or Sp2); red blood cells (RBCs; rich: RBC-R, poor: RBC-P); platelet concentration (PL: 2–3; PL: 4–6; PL: 6–8 and PL: 8–10 folds the baseline); leukocyte rich (Lc-R) or poor (Lc-P) and the range; activated (A+) or not (A−); light activated (L+) or not (L−), and injected with imaging guidance (G+) or not (G−) [35]. The above classification shows well how heterogeneous this treatment method is. According to the latest classification from 2020 proposed by Kon et al., and accepted by many researchers by consensus, the PRP used in our study should be reported as: 210-14-00. The digits refer to the whole blood PLT concentration (where "2" means 200,000–300,000 platelets/µL), PLT concentration in PRP (where "10" means 1,000,000–1,100,000 platelets/µL), presence of RBC in PRP (where "1" means $> 1 \times 10^6$/µL), increase in WBC from baseline (where "4" means 4.1–5.0 × baseline), presence of activation (where "0" means no external activation), and the addition of calcium (where "0" means no addition), respectively [37].

Several investigations were conducted to evaluate the content of the biologically active compounds in PRP obtained by various methods [19]. Significant positive correlations

were found between PLT concentrations in PRP and EGF, VEGF, PDGF, TGF-β1 [20,38,39] and these and other growth factors and cytokines are involved in the proper healing of soft tissues. They interact through stimulation of the migration of cells such as neutrophils, monocytes, and fibroblasts to the site of injury (PDGF), stimulation of extracellular matrix production (PDGF), stimulation of cell migration, proliferation, and differentiation (FGF, EGF, HGF, TGF-β1), as well as stimulation of angiogenesis (VEGF, HGF) [12,40,41]. In our study, a positive relationship between the above growth factors and clinical improvement was found with the EGF, VEGF, and PDGF, which could support their positive effect on the healing processes in vivo. Despite expectations, no relationship was found between any clinical outcome and TGF-β1. Inflammatory cytokines also could positively influence tissue healing by the attraction of macrophages and neutrophils (IL-8, MCP-1), the stimulation of reepithelialization (IL-8), inhibition of inflammation and scar formation (IL-10), stimulation of keratinocyte or fibroblast proliferation, and the regulation of immune responses (IL-1, IL-6, TNF-α) [12]. An increased amount of various inflammatory cytokines could also have a negative impact on tissue healing leading to an excessive inflammatory reaction. In our study, we found only one positive correlation between the inflammatory cytokine (IL-18) and clinical effect (increase in SEV) after one month, but it was no longer present after three months; however, some negative correlations were found between the clinical outcomes and IL10, IL-33 and IL-17A. Unfortunately, the numerous interactions between growth factors and cytokines in varying concentrations and proportions lead to almost unpredictable clinical effects inside the human body. Recent studies have demonstrated a significant role of IL-17 in the pathophysiology of tendinopathies. IL-17 has been shown to mediate the inflammatory response by stimulating the production of pro-inflammatory cytokines and tissue remodeling in human tenocytes. It is proposed as a potential therapeutic target in tendinopathy [42,43]. In our study, we found a negative correlation between IL-17A in the PRP and a functional improvement examined with the PRTEE questionnaire. This result may support the thesis that this cytokine plays an important role in lateral elbow tendinopathy.

Many in vitro, animal and human studies have been conducted to evaluate the effects of PRP on soft tissues and it has been effectively used for wound healing disorders, bone union disorders, sports injuries, osteoarthritis, rheumatoid arthritis and chronic overuse injuries such as different tendinopathies [44–46].

Niemiec et al., in their meta-analysis of 26 studies with PRP injection for lateral epicondylopathy, established the treatment as effective in the achievement of MCID during all points of follow-up (4–104 weeks) [16]. In another meta-analysis based on 16 studies, Chen et al., found that despite a significant clinical improvement, they were unable to recommend for or against the use of PRP for lateral epicondylitis. This was due to the small number of comparable studies, a lack of quantification of the specific PRP content, considerable heterogeneity between the randomized control trials, and most effect sizes being equivocal within the framework of two estimated MCID values [47]. Another point of view was presented by de Vos et al., in their systematic review based on six studies, in which the authors stated that there was strong evidence that PRP is not effective in treating chronic lateral elbow tendinosis [17]. Based on our experience and on the available literature, we believe that injection of PRP brings relief to a large number of patients with lateral elbow tendinopathy. Our findings lead to the assumption that a better result is expected when using a PRP with a higher platelet concentration. The use of preparations with a low content of platelets can be confusing and could be the reason for inconclusive results of systemic reviews and meta-analyses. On the other hand, research increasingly shows that there is no significant difference in the clinical effect between the different types of PRP [15]; therefore, it cannot be ruled out with certainty that the injection technique itself is of great importance in achieving clinical improvement [48]. Due to the lack of comparison with the control group, it cannot be excluded for sure, that the positive results of the PRP treatment in our study were related to the injection technique itself, a placebo-effect or to the recommended post-treatment rehabilitation protocol.

Only a few studies have been conducted to assess the correlation of growth factors in PRP with the clinical effect of the treatment. No previous study has assessed the correlation of inflammatory cytokines with clinical effect and to our knowledge, only one study has evaluated the correlation of the clinical effect of patients with tennis elbow treated with PRP injection and the growth factors within it. Lim et al., in their randomized controlled trial, evaluated 156 patients with lateral epicondylitis divided into two groups [49]. One group received a single injection of 2 ml of PRP and the control group was treated by physical therapy. For the final analysis, they evaluated 55 patients after receiving PRP injections and 50 in the control group. PDGF-AB, PDGF-BB, TGF-β, EGF, EGF, and IL-1b were among the growth factors assessed in PRP samples. During the follow-up at three and six months, pain intensity was evaluated with a VAS, and functional results with the Modified Mayo Clinic Performance Index. Additionally, a magnetic resonance imaging (MRI) was performed before treatment and after six months, to evaluate significant changes. At the final follow-up visit, VAS, MAYO, and MRI improvements were observed in both groups; however, it was significant only in the PRP group. No complications or adverse events were observed. The authors found a significant low correlation between the WBC and VAS improvement ($r = 0.318$) and an even lower correlation between the TGF-β and MAYO improvement ($r = 0.275$). Additionally, some positive significant correlations between an MRI improvement and both VEGF and TGF-β were reported [49]. As in our study, positive treatment results were obtained and positive correlations between the clinical outcomes and the content of the biologically active compounds in PRP were found; however, the authors showed a correlation between the treatment efficacy and TGF-β, which our study did not confirm. There were some small differences between the PRP used in our and their study. Similarly to our study, the authors used leukocyte-rich platelet-rich plasma, but they obtained a final mean platelet concentration approximately 6.87 times higher than the baseline compared to our 4.38. They also used 10% calcium chloride for the platelet activation just before injection. This kind of activation is responsible for a more rapid release of growth factors from the platelet α-granules. We decided to use natural platelet activation in vivo, by the contact with the native collagen present in the connective tissue for a more stable cytokine release [50]. Similar to our study, they also used approximately 2 ml of 1% lidocaine and administered a single injection into the tendon attachment. In their study, the VAS improvement at the final follow-up was higher than in our study (with a decrease in pain about 4.06 points in VAS recalculated to a 0–10 scale, compared to our 2.7 points). This could have been influenced by the longer observation period but the drop-outs in follow-up were not clearly stated in the publication. The functional improvement measured by the MAYO score was similar to those measured by the DASH and PRTEE in our study.

Another study that presented a relationship between the growth factors in PRP and the clinical outcome of its injection is the study published by Kim et al. [51]. The authors randomly divided 30 patients with rotator cuff tendinopathy into a group receiving a 2 mL injection of PRP or to a group undertaking only strengthening exercises. Pain and functional improvement were assessed with the American Shoulder and Elbow Surgeons (ASES), Constant–Murley score, and numeric rating scale (NRS) during 6, 12, and 24 weeks of follow-up. The PRP samples were analyzed to establish the concentrations of TGF-β1, TNF-α, PDGF-AA, PDGF-AB, PDGF-BB, VEGF, EGF, IGFBP-1, IL-1β, and IL-8. The authors found a significant correlation between cytokines and clinical outcomes only at 12 weeks of follow-up between the IL-1β and a change in the Constant–Murley score ($p = 0.046$), and between the TGF-β1 and a change in the NRS at 12 weeks ($p = 0.048$). The cut-off values to predict a meaningful improvement were established at 5.19 pg/mL in the IL-1β and 61.79 μg/mL in the TGF-β1. In their study, the authors concluded that better clinical outcomes for rotator cuff tendinopathy were found in patients who received PRP with IL-8 and TGF-β1 above these cut-off values than for the exercise group. The biggest limitation of this study was a relatively small group with a high percentage of drop-outs during the follow-up stages [51]. Again, our findings do not support a significant relationship

between IL-8 or TGF-β1 and clinical outcomes. This may, however, be due to significant methodological differences between the two studies.

Louis et al., showed results of a randomized blinded controlled trial comparing PRP and hyaluronic acid injection into osteoarthritic knees [52]. Fifty four patients were divided into comparable groups and were assessed by the Western Ontario and McMaster Universities Arthritis Index (WOMAC) score at a baseline and at one, three, and six months. The VEGF, PDGF-AB, and TGF-β1 contents of injected PRP were assessed. The authors found clinical improvement in both groups but also a correlation between a worsening in the WOMAC score and high concentrations in PRP of both TGF-β1 and PDGF-AB [52]. In our study, we found a positive correlation between the VEGF or PDGF concentrations in PRP and clinical outcomes; however, the results of osteoarthritis treatment cannot be directly translated into a treatment for tendinopathy due to significant differences in the pathophysiology of both diseases.

Rodrigues et al., in their randomized controlled trial, evaluated the relationship between growth factors in PRP and hair growth parameters on patients with alopecia treated with four subcutaneous PRP injections or a placebo [53]. PDGF, EGF and VEGF were measured in PRP samples. The authors demonstrated a significant increase in hair count, hair density and percentage of anagen hairs in the PRP group versus in the control group, without any correlation with platelet or growth factor concentrations in the PRP [53].

In our study, we also found a positive correlation between symptoms' severity at the beginning, and the size of improvement in both pain intensity and functional outcomes. Haahr et al., tried to assess the prognostic factors of 266 lateral epicondylitis cases treated with minimal occupational intervention during a one-year follow-up. They found that a poor prognosis was related to manual work and a high baseline pain [4]. In contrast, in our study, the greater the pain complaints were at the baseline, the greater the improvement that was obtained. This may lead to the assumption that patients with low severity will benefit more from a rehabilitation program, and those with symptoms of significant severity will benefit more from the earlier implementation of injection therapy.

The strengths of our study include a wide spectrum of clinical evaluation of the PRP injection treatment efficacy in patients with lateral elbow enthesopathy. That includes an assessment of the mean pain for the current day, pain intensity during provocation tests, tenderness of the affected area, the strength of various muscle groups and a functional assessment using questionnaires. The wide range of growth factors and inflammatory cytokines analyzed far exceeds the amount presented in other studies. The relationship of most of the studied molecules in PRP and their clinical effect was investigated for the first time.

The limitations of the study include the relatively small group of patients, the lack of MRI control before and after treatment, and not considering some of the existing cytokines and growth factors that may interfere with the healing process. The study shows the correlation of clinical outcomes and individual growth factors or inflammatory cytokines, but does not take into account the possibility of their mutual influence, both stimulating and inhibiting. Unfortunately, much larger groups of subjects would be needed to reliably estimate the clinical efficacy of the various combinations of cytokines. Another limitation of the presented study is the lack of results from the control groups. These will be provided after the long-term end of further data collection. Additionally, the study has a relatively short observation time; however, according to our experience and the experience of other researchers, waiting for any clinical pain improvement for more than three months is often unacceptable for patients who start seeking help elsewhere and often drop out of the study [18].

5. Conclusions

The injection of autologous platelet-rich plasma is a safe and effective treatment method for patients with lateral elbow tendinopathy. Our findings showed that PRP with a higher platelet concentration is correlated with a better decrease in pain during

three months of follow-up. For the first time in the literature, we showed a significant positive correlation of EGF, VEGF and PDGF concentrations in PRP and the size of pain reduction among those patients. After three months, the EGF concentration in the PRP significantly correlated with an improvement in grip strength, strength of wrist extension and size of functional improvement measured by a PRTEE. Negative correlations were found between the IL-10, IL-33 and IL-17A concentrations in the PRP and the change in the functional questionnaires in the third month after intervention. Further studies are needed in a larger group, with a longer follow-up, other tendinopathies, and with the use of different types of PRP to reliably assess the relationship between the clinical effect and the content of the biologically active compounds in injected PRP.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm11133687/s1, Table S1: Growth factors and inflammatory cytokines concentrations in all PRP samples [pg/mL]; Table S2: Outcome measurements before (0) and during follow-up (1, and 3 months).

Author Contributions: Conceptualization, M.D. and P.R.; methodology, M.D., P.R., E.B. and A.K.; formal analysis, M.D., E.B. and A.K.; investigation, M.D., H.M., S.P. and P.R.; resources, M.D., H.M. and S.P.; data curation, M.D.; writing—original draft preparation, M.D.; writing—review and editing, E.B., P.R. and A.K.; visualization, M.D.; supervision, A.K.; project administration, P.R.; funding acquisition, M.D. and P.R. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the grant from the project entitled "Wroclaw Medical University as a Regional Center of Excellence in the field of medical sciences and health sciences" implemented under the funds of the Ministry of Science and Higher Education (Republic of Poland) in the program "Regional Initiative of Excellence" in the years 2019–2022, project number 016/RID/2018/19, (grant number RID.Z501.20.008).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of Wroclaw Medical University (KB–26/2019, 21 January 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data used to support the findings of this study are available in the Supplementary Files. All data that may violate the ethics or privacy of subjects are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Kheiran, A.; Pandey, A.; Pandey, R. Common tendinopathies around the elbow; what does current evidence say? *J. Clin. Orthop. Trauma* **2021**, *19*, 216–223. [CrossRef] [PubMed]
2. Shiri, R.; Viikari-juntura, E.; Varonen, H.; Heliо, M. Prevalence and Determinants of Lateral and Medial Epicondylitis: A Population Study. *Am. J. Epidemiol.* **2006**, *164*, 1065–1074. [CrossRef] [PubMed]
3. Stasinopoulos, D.; Johnson, M.I. 'Lateral elbow tendinopathy' is the most appropriate diagnostic term for the condition commonly referred-to as lateral epicondylitis. *Med. Hypotheses* **2006**, *67*, 1400–1402. [CrossRef] [PubMed]
4. Haahr, J.P. Prognostic factors in lateral epicondylitis: A randomized trial with one-year follow-up in 266 new cases treated with minimal occupational intervention or the usual approach in general practice. *Rheumatology* **2003**, *42*, 1216–1225. [CrossRef]
5. Degen, R.M.; Conti, M.S.; Camp, C.L.; Altchek, D.W.; Dines, J.S.; Werner, B.C. Epidemiology and Disease Burden of Lateral Epicondylitis in the USA: Analysis of 85,318 Patients. *HSS J. Musculoskelet. J. Hosp. Spec. Surg.* **2018**, *14*, 9–14. [CrossRef]
6. Kraushaar, B.S.; Nirschl, R.P. Tendinosis of the Elbow (Tennis Elbow). Clinical features and findings of histological, immunohistochemical, and electron microscopy studies. *J. Bone Jt. Surg.* **1999**, *81*, 259–278. [CrossRef]
7. Longo, U.G.; Franceschetti, E.; Rizzello, G.; Petrillo, S.; Denaro, V. Elbow tendinopathy. *Muscles Ligaments Tendons J.* **2012**, *2*, 115–120. [CrossRef]

8. Lian, J.; Mohamadi, A.; Chan, J.J.; Hanna, P.; Hemmati, D.; Lechtig, A.; Nazarian, A. Comparative Efficacy and Safety of Nonsurgical Treatment Options for Enthesopathy of the Extensor Carpi Radialis Brevis A Systematic Review and Meta-analysis of Randomized Placebo-Controlled Trials. *Am. J. Sports Med.* **2018**, *47*, 3019–3029. [CrossRef]
9. Smidt, N.; van der Windt, D.A.; Assendelft, W.J.; Devillé, W.L.; Korthals-de Bos, I.B.; Bouter, L.M. Corticosteroid injections, physiotherapy, or a wait-and-see policy for lateral epicondylitis: A randomised controlled trial. *Lancet* **2002**, *359*, 657–662. [CrossRef]
10. Coombes, B.K.; Bisset, L.; Vicenzino, B. Efficacy and safety of corticosteroid injections and other injections for management of tendinopathy: A systematic review of randomised controlled trials. *Lancet* **2010**, *376*, 1751–1767. [CrossRef]
11. Dhurat, R.; Sukesh, M. Principles and methods of preparation of platelet-rich plasma: A review and author's perspective. *J. Cutan. Aesthet. Surg.* **2014**, *7*, 189. [CrossRef] [PubMed]
12. Werner, S.; Grose, R. Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* **2003**, *83*, 835–870. [CrossRef]
13. Watts, A.C.; Morgan, B.W.; Birch, A.; Nuttall, D.; Trail, I.A. Comparing leukocyte-rich platelet-rich plasma injection with surgical intervention for the management of refractory tennis elbow. A prospective randomised trial. *Shoulder Elb.* **2020**, *12*, 46–53. [CrossRef] [PubMed]
14. Simental-Mendía, M.; Vilchez-Cavazos, F.; Álvarez-Villalobos, N.; Blázquez-Saldaña, J.; Peña-Martínez, V.; Villarreal-Villarreal, G.; Acosta-Olivo, C. Clinical efficacy of platelet-rich plasma in the treatment of lateral epicondylitis: A systematic review and meta-analysis of randomized placebo-controlled clinical trials. *Clin. Rheumatol.* **2020**, *39*, 2255–2265. [CrossRef]
15. Muthu, S.; Patel, S.; Selvaraj, P.; Jeyaraman, M. Comparative analysis of leucocyte poor vs. leucocyte rich platelet-rich plasma in the management of lateral epicondylitis: Systematic review & meta-analysis of randomised controlled trials. *J. Clin. Orthop. Trauma* **2021**, *19*, 96–107. [CrossRef]
16. Niemiec, P.; Szyluk, K.; Jarosz, A.; Iwanicki, T.; Balcerzyk, A. Effectiveness of Platelet-Rich Plasma for Lateral Epicondylitis: A Systematic Review and Meta-analysis Based on Achievement of Minimal Clinically Important Difference. *Orthop. J. Sports Med.* **2022**, *10*, 232596712210869. [CrossRef] [PubMed]
17. de Vos, R.-J.; Windt, J.; Weir, A. Strong evidence against platelet-rich plasma injections for chronic lateral epicondylar tendinopathy: A systematic review. *Br. J. Sports Med.* **2014**, *48*, 952–956. [CrossRef] [PubMed]
18. Krogh, T.P.; Fredberg, U.; Stengaard-Pedersen, K.; Christensen, R.; Jensen, P.; Ellingsen, T. Treatment of lateral epicondylitis with platelet-rich plasma, glucocorticoid, or saline: A randomized, double-blind, placebo-controlled trial. *Am. J. Sports Med.* **2013**, *41*, 625–635. [CrossRef]
19. Oudelaar, B.W.; Peerbooms, J.C.; Huis in 't Veld, R.; Vochteloo, A.J.H. Concentrations of Blood Components in Commercial Platelet-Rich Plasma Separation Systems: A Review of the Literature. *Am. J. Sports Med.* **2019**, *47*, 479–487. [CrossRef]
20. Dejnek, M.; Moreira, H.; Płaczkowska, S.; Morasiewicz, P.; Barg, E.; Witkowski, J.; Reichert, P. Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study. *Adv. Clin. Exp. Med.* **2021**, *30*, 757–764. [CrossRef]
21. Krogh, T.; Fredberg, U.; Christensen, R.; Stengaard-Pedersen, K.; Ellingsen, T. Ultrasonographic Assessment of Tendon Thickness, Doppler Activity and Bony Spurs of the Elbow in Patients with Lateral Epicondylitis and Healthy Subjects: A Reliability and Agreement Study. *Ultraschall Med.* **2013**, *34*, 468–474. [CrossRef] [PubMed]
22. Zimmermann, R.; Arnold, D.; Strasser, E.; Ringwald, J.; Schlegel, A.; Wiltfang, J.; Eckstein, R. Sample preparation technique and white cell content influence the detectable levels of growth factors in platelet concentrates. *Vox Sang.* **2003**, *85*, 283–289. [CrossRef] [PubMed]
23. Gupta, P.K.; Acharya, A.; Khanna, V.; Roy, S.; Khillan, K.; Sambandam, S.N. PRP versus steroids in a deadlock for efficacy: Long-term stability versus short-term intensity—Results from a randomised trial. *Musculoskelet. Surg.* **2020**, *104*, 285–294. [CrossRef] [PubMed]
24. Beaton, D.E.; Katz, J.N.; Fossel, A.H.; Wright, J.G.; Tarasuk, V.; Bombardier, C. Measuring the whole or the parts? Validity, reliability, and responsiveness of the Disabilities of the Arm, Shoulder and Hand outcome measure in different regions of the upper extremity. *J. Hand Ther.* **2001**, *14*, 128–146. [CrossRef]
25. Rompe, J.D.; Overend, T.J.; MacDermid, J.C. Validation of the Patient-rated Tennis Elbow Evaluation Questionnaire. *J. Hand Ther.* **2007**, *20*, 3–11. [CrossRef]
26. Golicki, D.; Krzysiak, M.; Strzelczyk, P. Translation and Cultural Adaptation of the Polish Version of "Disabilities of the Arm, Shoulder and Hand" (DASH) and Quickdash Questionnaires. *Value Health* **2013**, *16*, A602. [CrossRef]
27. Doguł, P.; Latosiewicz, R.; Goguł, M.; Majewska, D.; Gawęda, K. Jakość tłumaczenia polskiego i adaptacji kulturowej kwestionariusza PRTEE (Patient—Rated Tennis Elbow Evaluation). *J. Educ. Health Sport* **2016**, *6*, 424–435.
28. Schneeberger, A.G.; Kösters, M.C.; Steens, W. Comparison of the subjective elbow value and the Mayo Elbow Performance Score. *J. Shoulder Elb. Surg.* **2014**, *23*, 308–312. [CrossRef]
29. Marx, R.E. Platelet-rich plasma (PRP): What is PRP and what is not PRP? *Implant Dent.* **2001**, *10*, 225–228. [CrossRef]
30. Mazzucco, L.; Balbo, V.; Cattana, E.; Guaschino, R.; Borzini, P. Not every PRP-gel is born equal Evaluation of growth factor availability for tissues through four PRP-gel preparations: Fibrinet®, RegenPRP-Kit®, Plateltex® and one manual procedure. *Vox Sang.* **2009**, *97*, 110–118. [CrossRef]
31. Kobayashi, Y.; Saita, Y.; Nishio, H.; Ikeda, H.; Takazawa, Y.; Nagao, M.; Takaku, T.; Komatsu, N.; Kaneko, K. Leukocyte concentration and composition in platelet-rich plasma (PRP) influences the growth factor and protease concentrations. *J. Orthop. Sci.* **2016**, *21*, 683–689. [CrossRef] [PubMed]

32. Cieślik-Bielecka, A.; Reichert, P.; Skowroński, R.; Królikowska, A.; Bielecki, T. A new aspect of in vitro antimicrobial leukocyte- and platelet-rich plasma activity based on flow cytometry assessment. *Platelets* **2019**, *30*, 728–736. [CrossRef] [PubMed]
33. Dohan Ehrenfest, D.M.; Rasmusson, L.; Albrektsson, T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol.* **2009**, *27*, 158–167. [CrossRef] [PubMed]
34. Mishra, A.; Harmon, K.; Woodall, J.; Vieira, A. Sports Medicine Applications of Platelet Rich Plasma. *Curr. Pharm. Biotechnol.* **2012**, *13*, 1185–1195. [CrossRef]
35. Lana, J.F.S.D.; Purita, J.; Paulus, C.; Huber, S.C.; Rodrigues, B.L.; Rodrigues, A.A.; Santana, M.H.; Madureira, J.L.; Malheiros Luzo, Â.C.; Belangero, W.D.; et al. Contributions for classification of platelet rich plasma—Proposal of a new classification: MARSPILL. *Regen. Med.* **2017**, *12*, 565–574. [CrossRef]
36. Murray, I.R.; Geeslin, A.G.; Goudie, E.B.; Petrigliano, F.A.; LaPrade, R.F. Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO). *J. Bone Jt. Surg.* **2017**, *99*, 809–819. [CrossRef]
37. Kon, E.; Di Matteo, B.; Delgado, D.; Cole, B.J.; Dorotei, A.; Dragoo, J.L.; Filardo, G.; Fortier, L.A.; Giuffrida, A.; Jo, C.H.; et al. Platelet-rich plasma for the treatment of knee osteoarthritis: An expert opinion and proposal for a novel classification and coding system. *Expert. Opin. Biol. Ther.* **2020**, *20*, 1447–1460. [CrossRef]
38. Sundman, E.A.; Cole, B.J.; Fortier, L.A. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am. J. Sports Med.* **2011**, *39*, 2135–2140. [CrossRef]
39. Magalon, J.; Bausset, O.; Serratrice, N.; Giraudo, L.; Aboudou, H.; Veran, J.; Magalon, G.; Dignat-Georges, F.; Sabatier, F. Characterization and comparison of 5 platelet-rich plasma preparations in a single-donor model. *Arthrosc.—J. Arthrosc. Relat. Surg.* **2014**, *30*, 629–638. [CrossRef]
40. Sánchez-González, D.J.; Méndez-Bolaina, E.; Trejo-Bahena, N.I. Platelet-rich plasma peptides: Key for regeneration. *Int. J. Pept.* **2012**, *2012*, 532519. [CrossRef]
41. Molloy, T.; Wang, Y.; Murrell, G.A.C. The roles of growth factors in tendon and ligament healing. *Sports Med.* **2003**, *33*, 381–394. [CrossRef] [PubMed]
42. Mimpen, J.Y.; Snelling, S.J.B.; Carr, A.J.; Dakin, S.G. Interleukin-17 Cytokines and Receptors: Potential Amplifiers of Tendon Inflammation. *Front. Bioeng. Biotechnol.* **2021**, *9*, 795830. [CrossRef] [PubMed]
43. Millar, N.L.; Akbar, M.; Campbell, A.L.; Reilly, J.H.; Kerr, S.C.; McLean, M.; Frleta-Gilchrist, M.; Fazzi, U.G.; Leach, W.J.; Rooney, B.P.; et al. IL-17A mediates inflammatory and tissue remodelling events in early human tendinopathy. *Sci. Rep.* **2016**, *6*, 27149. [CrossRef] [PubMed]
44. Saif, D.S.; Hegazy, N.N.; Zahran, E.S. Evaluating the efficacy of intra-articular injections of platelet rich plasma (PRP) in rheumatoid arthritis patients and its impact on inflammatory cytokines, disease activity and quality of life. *Curr. Rheumatol. Rev.* **2020**, *16*, 232–241. [CrossRef] [PubMed]
45. Tietze, D.C.; Geissler, K.; Borchers, J. The effects of platelet-rich plasma in the treatment of large-joint osteoarthritis: A systematic review. *Phys. Sportsmed.* **2014**, *42*, 27–37. [CrossRef]
46. Hussain, N.; Johal, H.; Bhandari, M. An evidence-based evaluation on the use of platelet rich plasma in orthopedics—A review of the literature. *SICOT-J.* **2017**, *3*, 57. [CrossRef]
47. Chen, X.T.; Fang, W.; Jones, I.A.; Heckmann, N.D.; Park, C.; Vangsness, C.T. The Efficacy of Platelet-Rich Plasma for Improving Pain and Function in Lateral Epicondylitis: A Systematic Review and Meta-analysis with Risk-of-Bias Assessment. *Arthrosc. J. Arthrosc. Relat. Surg.* **2021**, *37*, 2937–2952. [CrossRef]
48. Gao, B.; Dwivedi, S.; DeFroda, S.; Bokshan, S.; Ready, L.V.; Cole, B.J.; Owens, B.D. The Therapeutic Benefits of Saline Solution Injection for Lateral Epicondylitis: A Meta-analysis of Randomized Controlled Trials Comparing Saline Injections with Nonsurgical Injection Therapies. *Arthrosc. J. Arthrosc. Relat. Surg.* **2019**, *35*, 1847–1859.e12. [CrossRef]
49. Lim, W.; Park, S.H.; Kim, B.; Kang, S.W.; Lee, J.W.; Moon, Y.L. Relationship of cytokine levels and clinical effect on platelet-rich plasma-treated lateral epicondylitis. *J. Orthop. Res.* **2018**, *36*, 913–920. [CrossRef]
50. Cavallo, C.; Roffi, A.; Grigolo, B.; Mariani, E.; Pratelli, L.; Merli, G.; Kon, E.; Marcacci, M.; Filardo, G. Platelet-Rich Plasma: The Choice of Activation Method Affects the Release of Bioactive Molecules. *BioMed Res. Int.* **2016**, *2016*, 6591717. [CrossRef]
51. Kim, S.J.; Yeo, S.M.; Noh, S.J.; Ha, C.-W.; Lee, B.C.; Lee, H.S.; Kim, S.J. Effect of platelet-rich plasma on the degenerative rotator cuff tendinopathy according to the compositions. *J. Orthop. Surg. Res.* **2019**, *14*, 408. [CrossRef] [PubMed]
52. Louis, M.L.; Magalon, J.; Jouve, E.; Bornet, C.E.; Mattei, J.C.; Chagnaud, C.; Rochwerger, A.; Veran, J.; Sabatier, F. Growth Factors Levels Determine Efficacy of Platelets Rich Plasma Injection in Knee Osteoarthritis: A Randomized Double Blind Noninferiority Trial Compared with Viscosupplementation. *Arthrosc. J. Arthrosc. Relat. Surg.* **2018**, *34*, 1530–1540.e2. [CrossRef] [PubMed]
53. Rodrigues, B.L.; Montalvão, S.A.L.; Cancela, R.B.B.; Silva, F.A.R.; Urban, A.; Huber, S.C.; Júnior, J.L.R.C.; Lana, J.F.S.D.; Annichinno-Bizzacchi, J.M. Treatment of male pattern alopecia with platelet-rich plasma: A double-blind controlled study with analysis of platelet number and growth factor levels. *J. Am. Acad. Dermatol.* **2019**, *80*, 694–700. [CrossRef] [PubMed]

Supplementary Table S1. Growth factors and inflammatory cytokines concentrations in all PRP samples [pg/ml].

	Mean	SD	Median	Q1 – Q3
Transforming Growth Factor-β1, free active	382.46	256.01	337.36	257.65 – 443.32
Epidermal growth factor	282.77	263.27	219.13	116.43 – 340.91
Fibroblast Growth Factor- basic	2826.10	8119.49	315.98	254.70 – 450.50
Vascular endothelial growth factor	384.69	487.88	197.10	40.95 – 602.85
Hepatocyte growth factor	497.65	1027.87	208.98	156.05 – 290.69
Platelet-Derived Growth Factor-AA	132665.17	54524.17	140920.85	90062.55 – 183956.91
Platelet-Derived Growth Factor-BB	49160.61	18377.38	52190.81	29826.86 – 62573.63
Interleukin-1β	76.16	70.67	46.69	30.61 – 100.44
Interferon-α2	39.76	28.85	27.13	16.16 – 50.14
Interferon-γ	7.10	3.73	4.87	4.87 – 7.45
Tumor Necrosis Factor α	33.76	27.29	23.81	13.02 – 42.72
Monocyte Chemoattractant Protein-1	128.18	125.01	94.22	63.97 – 151.18
Interleukin-6	20.23	12.05	15.24	12.16 – 22.34
Interleukin-8	126.66	133.63	61.38	46.77 – 157.73
Interleukin-10	20.10	9.78	17.28	12.88 – 24.98
Interleukin-12p70	21.09	11.33	16.78	11.47 – 27.80
Interleukin-17A	3.22	1.70	2.58	1.94 – 4.28
Interleukin-18	411.35	293.41	334.12	185.68 – 507.57
Interleukin-23	77.14	24.03	65.97	65.97 – 65.97
Interleukin-33	177.51	100.79	161.02	95.95 – 229.86

PRP: Platelet-rich plasma; SD: standard deviation; Q1 – Q3: the first and the third quartile.

Supplementary Table S2. Outcome measurements before (0) and during follow-up (1, 3 month).

	Mean	SD	Median	Q1 – Q3	*p (vs. 0)*
0 VAS	5.07	1.76	5.00	4 – 6	
1 VAS	3.2	1.88	3.00	2 – 4	≤ 0.001
3 VAS	2.37	2.12	2.00	1 – 3	≤ 0.001
0 Cozen's test	5.42	2.55	5.00	5 – 7.5	
1 Cozen's test	3.33	2.21	2.5	2.5 – 5.0	≤ 0.001
3 Cozen's test	1.95	2.58	0.5	0 – 2.5	≤ 0.001
0 Mill's test	4.25	3.09	5.00	2.5 – 7.5	
1 Mill's test	2.08	2.37	2.5	0 – 2.5	≤ 0.001
3 Mill's test	1.2	1.99	0	0 – 2.5	≤ 0.001
0 Maudsley's test	2.67	2.54	2.50	0 – 5.0	
1 Maudsley's test	1.33	1.83	0	0 – 2.5	< 0.05
3 Maudsley's test	0.95	1.90	0	0 – 1.0	< 0.05
0 Maudsley's test in extended elbow	6.42	2.24	7.50	5 – 7.5	
1 Maudsley's test in extended elbow	4.83	2.62	5.0	2.5 – 7.5	< 0.05
3 Maudsley's test in extended elbow	3.4	2.45	2.5	1.5 – 5.0	≤ 0.001
0 Thomson's test	7.5	2.18	7.5	7.5 – 10	
1 Thomson's test	6	2.50	7.5	5 – 7.5	< 0.05
3 Thomson's test	3.73	2.5	4.00	2.5 – 5.0	≤ 0.001
0 Chair test	6	2.24	6.25	5 – 7.5	
1 Chair test	4.83	2.62	5.00	2.5 – 7.5	< 0.05
3 Chair test	2.65	2.63	2.5	0 – 5.0	≤ 0.001
0 PPT [N]	25.38	11.76	22.21	18.14 – 33.15	
1 PPT [N]	28.06	11.81	25.20	20.69 – 30.60	0.102
3 PPT [N]	30.44	14.28	28.39	21.67 – 34.13	< 0.05
0 SEV	47.50	17.36	50	40 – 50	
1 SEV	67.17	14.84	70	60 – 80	≤ 0.001
3 SEV	73.77	21.04	80	70 – 90	≤ 0.001
0 DASH	37.28	17.17	33.75	22.5 – 47.50	
1 DASH	24.78	16.04	18.75	12.5 – 36.67	
3 DASH	15.17	12.32	12.08	7.5 – 17.50	≤ 0.001
0 PRTEE	45.98	19.77	41.25	32.5 – 52.00	
1 PRTEE	28.73	17.37	23.25	17.5 – 39.50	≤ 0.001
3 PRTEE	18.71	16.30	13.5	9 – 21.00	≤ 0.001
0 grip strength	32.5	14.28	30.0	22.00 – 44.0	
1 grip strength	33.4	13.63	30.0	22.00 – 46.0	0,728
3 grip strength	35.8	15.13	36.0	22.00 – 50.0	< 0.05
0 wrist extension strength	116,4	52,47	109,3	78,45 – 158.9	
1 wrist extension strength	121,5	51,14	107,9	90,22 – 151.0	0.804
3 wrist extension strength	140,6	50,01	148,1	101,01 – 177.5	< 0.05

0 wrist flexion strength	159,0	61,28	146,6	118,66 – 199.1	
1 wrist flexion strength	151,8	48,27	141,7	116,70 – 193.2	0.304
3 wrist flexion strength	162,7	58,37	154,9	114,74 – 200.1	0.501
0 forearm supination strength	26,1	12,13	23,5	16,67 – 32.4	
1 forearm supination strength	24,7	10,54	23,5	17,65 – 33.3	0.311
3 forearm supination strength	29,8	16,19	23,5	18,63 – 36.3	0.221
0 forearm pronation strength	36,6	18,35	27,9	24,52 – 50.0	
1 forearm pronation strength	40,2	17,93	33,3	26,48 – 55.9	0.094
3 forearm pronation strength	47,6	20,46	42,2	28,44 – 62.8	≤ 0.001
0 elbow extension strength	176,0	66,71	160,3	128,47 – 215.7	
1 elbow extension strength	172,1	54,51	155,4	129,45 – 207.9	0.433
3 elbow extension strength	170,2	58,46	155,9	125,53 – 217.7	0.600
0 elbow flexion strength	235,0	90,34	220,2	147,10 – 302.0	
1 elbow flexion strength	236,8	91,14	208,4	165,73 – 313.8	0.805
3 elbow flexion strength	238,7	79,80	215,7	170,64 – 317.7	< 0.05

PPT: Pressure Pain Threshold; SD: standard deviation; Q1 – Q3: the first and the third quartile; SEV: Subjected Elbow Value; DASH: Disability of Arm, Shoulder and Hand Questionnaire; PRTEE: Patient-rated tennis elbow evaluation.

VII. STRESZCZENIE

Wstęp

Entezopatia nadkłykcia bocznego kości ramiennej, powszechnie znana jako „łokieć tenisisty" jest częstym schorzeniem znacznie utrudniającym codzienne funkcjonowanie. W przypadku gdy odpoczynek, leki przeciwbólowe, ćwiczenia i fizykoterapia nie przyniosą oczekiwanej ulgi, choroba może przeistoczyć się w proces przewlekły trwający od kilku miesięcy do kilku lat. Aktualnie nie istnieje jedna skuteczna terapia, która przyniosłaby szybką pomoc takim pacjentom. Jedną z wielu proponowanych metod leczenia jest iniekcja autologicznego osocza bogatopłytkowego (PRP). Jej istotą jest dostarczenie do zmienionej chorobowo tkanki wysokiego stężenia czynników wzrostu zawartych w płytkach krwi, które poprzez swoje oddziaływanie parakrynne stymulujące procesy regeneracyjne. Istnieją liczne kontrowersje związane ze skutecznością tej metody wynikające często ze sprzecznych wyników badań klinicznych. Do przyczyn tego stanu można zaliczyć zarówno różnice w składzie komórkowym i molekularnym osocza poszczególnych pacjentów jak i różnice w sposobach przygotowywania PRP, co w konsekwencji może powodować uzyskanie preparatów o zupełnie innej zawartości składników biologicznie aktywnych.

Cel pracy

Celem powyższej pracy doktorskiej było ustalenie czy istnieje zależność pomiędzy zawartością składników biologicznie aktywnych w PRP, takich jak składniki komórkowe i molekularne (czynniki wzrostu, cytokiny zapalne) a skutecznością kliniczną leczenia iniekcyjnego z jego pomocą pacjentów z entezopatią nadkłykcia bocznego kości ramiennej. Dodatkowo za cel obrano ocenę różnic zawartości składników biologicznie aktywnych w preparatach PRP przygotowanych z użyciem różnych dostępnych metod.

Materiał i metody

W pierwszym etapie wykonano badania porównujące zawartość składu komórkowego oraz wybranych czynników wzrostu i cytokin zapalnych w próbkach PRP przygotowanych z użyciem czterech różnych metod. Analizy stężenia cząsteczek parakrynnych dokonano z wykorzystaniem immunofluorescencyjnej metody multipleksowej LEGENDplex™ (BioLegend, USA) i cytometrii przepływowej. Na podstawie badań przedklinicznych wytypowano najbardziej optymalną metodę pozyskiwania PRP. W kolejnym etapie wykorzystano PRP w ramach iniekcyjnego leczenia 30 pacjentów z entezopatią nadkłykcia bocznego kości ramiennej. Próbki krwi każdego pacjenta oraz uzyskanego z nich PRP poddano analizie laboratoryjnej na zawartość składników biologicznie aktywnych. Skuteczność leczenia oceniano poprzez określenie zmiany natężenia dolegliwości bólowych w skali VAS i badaniem algometrycznym, zmianę siły wybranych grup mięśniowych kończyny górnej w badaniu dynamometrycznym jak również zmiany w punktacji uzyskanej w kwestionariuszach funkcjonalnych (Disability of Arm, Shoulder and Hand (DASH), Patient Rated Tennis Elbow Evaluation (PRTEE)). W trakcie analizy statystycznej ustalono różnice ilościowe i jakościowe pomiędzy zawartością składników biologicznie aktywnych we krwi pacjentów oraz uzyskanym z niej PRP, a także określono ich zależność w stosunku do skuteczności klinicznej leczenia pacjentów w trakcie 3 miesięcznej obserwacji.

Wyniki

W próbkach PRP przygotowanych z użyciem czterech różnych komercyjnych zestawów wykazano istotne różnice w zawartości płytek krwi, białych i czerwonych krwinek, czynników wzrostu, cytokin zapalnych oraz powtarzalności uzyskiwanych zagęszczeń. Spośród badanych zestawów do dalszych badań wybrano Mini GPS III Platelet Concentration System (Biomet Inc., USA) jako zestaw pozwalający na pozyskiwanie PRP o powtarzalnie wysokiej zawartości płytek krwi (5-krotny wzrost stężenia w porównaniu do krwi pełnej) i czynników wzrostu. Pomiędzy próbkami osocza a przygotowanym z niego PRP stwierdzono występowanie istotnych różnic w zawartości pięciu z sześciu badanych czynników wzrostu oraz pięciu z trzynastu cytokin zapalnych. Stwierdzono również występowanie istotnych korelacji pomiędzy zawartością składników komórkowych a stężeniem części z badanych czynników wzrostu i cytokin zapalanych. W badaniu oceniającym skuteczność kliniczną leczenia entezopatii nadkłykcia bocznego kości ramiennej uzyskano poprawę funkcjonalną oraz redukcję dolegliwości bólowych (Minimal Clinically Important Difference) po 3 miesiącach u większości badanych pacjentów. Stwierdzono występowanie istotnej dodatniej korelacji pomiędzy wielkością uzyskanego zmniejszenia dolegliwości bólowych a zawartością w PRP płytek krwi, naskórkowego czynnika wzrostu (EGF), naczyniowo-śródbłonkowego czynnika wzrostu oraz płytkopochodnych czynników wzrostu. Stężenie EGF w PRP pozytywnie korelowało również z poprawą funkcjonalną mierzoną przy pomocy kwestionariusza PRTEE, poprawą siły uścisku oraz siły prostowników nadgarstka.

Wnioski

Uzyskane wyniki badań przedklinicznych potwierdziły występowanie istotnych różnic pomiędzy zawartością składników biologicznie aktywnych w PRP przygotowanym przy użyciu czterech różnych protokołów. Przeprowadzone analizy laboratoryjne wykazały również, że PRP przygotowane z pomocą dostępnych komercyjnie zestawów może stanowić efektywne źródło molekuł o działaniu parakrynnym biorących udział w fizjologicznych procesach naprawczych. W przeprowadzonym badaniu klinicznym iniekcje PRP okazały się skuteczną i bezpieczną metodą leczenia pacjentów z entezopatią nadkłykcia bocznego kości ramiennej. Wykazano również, iż zawartość płytek krwi oraz stężenie czynników wzrostu w zastosowanym do iniekcji PRP pozytywnie koreluje z uzyskaną poprawą kliniczną.

VIII. ABSTRACT

Introduction

Lateral humeral enthesopathy, widely known as "tennis elbow," is a common condition that significantly impairs daily functioning. When rest, pain medications, exercise, and physical therapy fail to bring the expected improvement, the condition can develop into a chronic process lasting from several months to several years. Currently, there is no single effective therapy that will bring quick relief to such patients. One of the many proposed treatment methods is the injection of autologous platelet-rich plasma (PRP). The essence of this method is to provide the affected tissue with a high concentration of growth factors contained in platelets, which by their paracrine action stimulate local regenerative processes. There are numerous controversies related to the effectiveness of this method, often resulting from contradictory results of clinical studies. The reasons for this state include differences in the cellular and molecular composition of plasma from individual patients and differences in the methods of PRP preparation, which may result in the final product with a completely different content of biologically active components.

Purpose of the study

The aim of this dissertation was to determine whether there is any correlation between the content of biologically active components in PRP, such as cells and molecules (growth factors, inflammatory cytokines), and the clinical efficacy of PRP-based injection treatment of patients with lateral humeral enthesopathy. The additional aim was to evaluate the differences in the content of biologically active components in PRP samples prepared with the use of different available methods.

Material and methods

In the first stage, we performed studies comparing the content of cellular composition, selected growth factors and inflammatory cytokines in PRP samples obtained using four different methods. The concentrations of paracrine molecules were analyzed using multiplex bead immunoassays that use fluorescence-encoded beads LEGENDplex™ (BioLegend, USA) and flow cytometer measurements. Based on preclinical studies, the most optimal method for obtaining PRP was selected. In the next step, PRP was used for the injection treatment of 30 patients with lateral humeral enthesopathy. Blood samples from each patient and the PRP obtained from them were analyzed for the content of biologically active components. The efficacy of the treatment was evaluated by determining the change in pain intensity according to VAS scale and algometric examination, the change in strength of selected muscle groups of the upper limb according to dynamometric examination, and the change in scores obtained in functional questionnaires (Disability of Arm, Shoulder and Hand (DASH) and Patient-Rated Tennis Elbow Evaluation (PRTEE)). Statistical analysis was performed to determine quantitative and qualitative differences between the content of biologically active components in the patients' blood and the PRP obtained from it and to determine their relation to the clinical efficacy of the patients' treatment during the 3- month follow-up.

Results

PRP samples prepared using four various commercial systems showed significant differences in the content of platelets, white and red blood cells, growth factors, inflammatory cytokines and repeatability of the obtained concentrations. Among the tested kits, the Mini GPS III Platelet Concentration System (Biomet Inc., USA) was selected for further studies as a system that allows to obtain PRP with a repetitively high concentration of platelets (5 times compared to the baseline of whole blood) and growth factors. Significant differences in the content of five out of six growth factors tested and five out of thirteen inflammatory cytokines were found between plasma samples and the PRP prepared from them. Significant correlations were also found between the content of cellular components and the concentration of some of the growth factors and inflammatory cytokines tested. In a study that evaluated the clinical efficacy of lateral humeral enthesopathy treatment, functional improvement and pain reduction (Minimal Clinically Important Difference) were observed after 3 months in the majority of patients. There was a significant positive correlation between the amount of pain reduction and the concentration of platelets, epidermal growth factor (EGF), vascular endothelial growth factor, and platelet-derived growth factors in PRP. The concentration of EGF in PRP was also positively correlated with the functional improvement measured by the PRTEE questionnaire, the improvement in grip strength and the strength of wrist extensors.

Conclusions

The results of the preclinical studies confirmed the existence of significant differences in the content of biologically active components in PRP prepared using four different protocols. Laboratory analyses also demonstrated that PRP prepared with commercially available kits may be an effective source of paracrine molecules involved in physiological repair processes. In the clinical trial, PRP injections proved to be an effective and safe treatment option for patients with lateral humeral enthesopathy. It has also been shown that the platelet content and the concentration of growth factors in the injected PRP positively correlate with clinical improvement.

IX. ZAŁĄCZNIKI

1. OŚWIADCZENIA WSPÓŁAUTORÓW

Wrocław, 22.08.2022

Maciej Dejnek
Katedra Chirurgii Urazowej,
Wydział Lekarski,
Wrocławski Uniwersytet Medyczny
ul. Borowska 213, 50-556 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na przygotowaniu planu badawczego, rekrutacji ochotników do badania, przygotowaniu i zabezpieczeniu materiału biologicznego do dalszej analizy laboratoryjnej, współudziale w przygotowaniu bazy danych, jej analiza i interpretacja, przygotowaniu pierwotnej wersji manuskryptu, przygotowaniu ilustracji, wprowadzeniu ostatecznych poprawek.

Maciej Dejnek

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na przygotowaniu planu badawczego, rekrutacji ochotników do badania, przygotowaniu i zabezpieczeniu materiału biologicznego do dalszej analizy laboratoryjnej, współudziale w przygotowaniu bazy danych, jej analiza i interpretacja, przygotowaniu pierwotnej wersji manuskryptu, przygotowaniu ilustracji, wprowadzeniu ostatecznych poprawek.

Maciej Dejnek

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses", Oxidative Medicine and Cellular Longevity, 2022:8059622 mój udział polegał na przygotowaniu planu badawczego, pozyskaniu środków finansowych, rekrutacji pacjentów do badania, przygotowaniu i zabezpieczeniu materiału biologicznego do dalszej analizy laboratoryjnej, współudziale w przygotowaniu bazy danych, jej analiza i interpretacja, przygotowaniu pierwotnej wersji manuskryptu, przygotowaniu ilustracji, wprowadzeniu ostatecznych poprawek.

Maciej Dejnek

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma", Journal of Clinical Medicine, 11(13):3687 mój udział polegał na współtworzeniu konceptu manuskryptu, przygotowaniu planu badawczego i metodologii badania, pozyskaniu środków finansowych, rekrutacji pacjentów do badania, ocena kliniczna i ultrasonograficzna pacjentów, przygotowaniu i zabezpieczeniu materiału biologicznego do dalszej analizy laboratoryjnej, współudziale w przygotowaniu bazy danych, jej analiza i interpretacja, przygotowaniu pierwotnej wersji manuskryptu, przygotowaniu ilustracji, wprowadzeniu ostatecznych poprawek.

Maciej Dejnek

Wrocław, 22.08.2022

Prof. dr hab. n. med. Paweł Reichert
Katedra Chirurgii Urazowej,
Wydział Lekarski,
Wrocławski Uniwersytet Medyczny
ul. Borowska 213, 50-556 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na stworzeniu konceptu manuskryptu, współudziale w tworzeniu planu badawczego, nadzorze merytorycznym nad projektem, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych, akceptacji ostatecznej wersji manuskryptu.

Uniwersytet Medyczny we Wrocławiu
KATEDRA CHIRURGII URAZOWEJ
KLINIKA CHIRURGII URAZOWEJ
I CHIRURGII RĘKI
kierownik
prof. dr hab. Paweł Reichert

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na współtworzeniu konceptu manuskryptu, współudziale w tworzeniu planu badawczego, nadzorze merytorycznym nad projektem, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Uniwersytet Medyczny we Wrocławiu
KATEDRA CHIRURGII URAZOWEJ
KLINIKA CHIRURGII URAZOWEJ
I CHIRURGII RĘKI
kierownik
prof. dr hab. Paweł Reichert

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses", Oxidative Medicine and Cellular Longevity, 2022:8059622 mój udział polegał na współtworzeniu konceptu manuskryptu, współudziale w tworzeniu planu badawczego, pozyskaniu środków finansowych, nadzorze merytorycznym nad częścią medyczną projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Uniwersytet Medyczny we Wrocławiu
KATEDRA CHIRURGII URAZOWEJ
KLINIKA CHIRURGII URAZOWEJ
I CHIRURGII RĘKI
Kierownik

prof. dr hab. Paweł Reichert

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma", Journal of Clinical Medicine, 11(13):3687 mój udział polegał na współtworzeniu konceptu manuskryptu, współudziale w tworzeniu planu badawczego i metodologii badania, pozyskaniu środków finansowych, prowadzeniu leczenia uczestników badania, nadzorze merytorycznym nad częścią medyczną projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Uniwersytet Medyczny we Wrocławiu
KATEDRA CHIRURGII URAZOWEJ
KLINIKA CHIRURGII URAZOWEJ
I CHIRURGII RĘKI
kierownik

prof. dr hab. Paweł Reichert

Wrocław, 22.08.2022

dr Helena Moreira
Katedra i Zakład Podstaw Nauk Medycznych,
Wydział Farmaceutyczny,
Wrocławski Uniwersytet Medyczny
ul. Borowska 211, 50-556, Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na przygotowaniu planu analiz biochemicznych, przeprowadzeniu analizy zawartości czynników wzrostu w materiale biologicznym pobranym od ochotników, współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej, przygotowanie części manuskryptu dotyczącej metodologicznych aspektów prowadzonych badań biochemicznych.

Helena Moreira

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na przeprowadzeniu analizy biochemicznej w materiale biologicznym pobranym od ochotników, współudziale w przygotowaniu bazy danych, jej analizy i interpretacji, przygotowanie części manuskryptu dotyczącej metodologicznych aspektów prowadzonych badań biochemicznych.

Helena Moreira

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses", Oxidative Medicine and Cellular Longevity, 2022:8059622 mój udział polegał na przeprowadzeniu analizy biochemicznej w materiale biologicznym pobranym od uczestników badania, współudziale w przygotowaniu bazy danych, jej analizy i interpretacji, przygotowanie części manuskryptu dotyczącej metodologicznych aspektów prowadzonych badań biochemicznych.

Helena Moreira

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma", Journal of Clinical Medicine, 11(13):3687 mój udział polegał na przeprowadzeniu analizy biochemicznej w materiale biologicznym pobranym od uczestników badania, współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej.

Helena Moreira

Wrocław, 22.08.2022

dr Sylwia Płaczkowska
Diagnostyczne Laboratorium Naukowo-Dydaktyczne,
Katedra Diagnostyki Laboratoryjnej,
Wydział Farmaceutyczny,
Wrocławski Uniwersytet Medyczny
ul. Borowska 211A, 50-556, Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na przeprowadzeniu analizy laboratoryjnej materiału biologicznego pobranego od ochotników, współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej.

Sylwia Płaczkowska

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na przeprowadzeniu analizy laboratoryjnej w materiale biologicznym pobranym od ochotników, współudziale w przygotowaniu bazy danych, jej analizy i interpretacji.

Sylwia Płaczkowska

Maciej Dejnek, Helena Moreira, Sylwia Płoczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses", Oxidative Medicine and Cellular Longevity, 2022:8059622 mój udział polegał na przeprowadzeniu analizy laboratoryjnej w materiale biologicznym pobranym od uczestników badania, współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej.

Maciej Dejnek, Helena Moreira, Sylwia Płoczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma", Journal of Clinical Medicine, 11(13):3687 mój udział polegał na przeprowadzeniu analizy laboratoryjnej w materiale biologicznym pobranym od uczestników badania, współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej.

Wrocław, 22.08.2022

dr hab. Aleksandra Królikowska
Samodzielna Pracownia Ergonomii i Monitoringu Biomedycznego,
Katedra Fizjoterapii,
Wydział Nauk o Zdrowiu,
Wrocławski Uniwersytet Medyczny
ul. Bartla 5, 51-618 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na współtworzeniu konceptu manuskryptu, współudziale w tworzeniu planu badawczego, nadzorze merytorycznym nad projektem, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych, akceptacji ostatecznej wersji manuskryptu.

Aleksandra Królikowska

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses", Oxidative Medicine and Cellular Longevity, 2022:8059622 mój udział polegał na współtworzeniu konceptu manuskryptu, nadzorze merytorycznym nad realizacją projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych, akceptacji ostatecznej wersji manuskryptu.

Aleksandra Królikowska

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma", Journal of Clinical Medicine, 11(13):3687 mój udział polegał na przygotowaniu planu badawczego i opracowaniu metodologii badania, nadzorze merytorycznym nad realizacją projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych, akceptacji ostatecznej wersji manuskryptu.

Aleksandra Królikowska

Wrocław, 22.08.2022

dr hab. Ewa Barg
Katedra i Zakład Podstaw Nauk Medycznych,
Wydział Farmaceutyczny,
Wrocławski Uniwersytet Medyczny
ul. Borowska 211, 50-556, Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na współtworzeniu konceptu oraz planu badawczego, nadzorze merytorycznym nad częścią laboratoryjną projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Leukocyte-rich platelet-rich plasma as an effective source of molecules that modulate local immune and inflammatory cell responses", Oxidative Medicine and Cellular Longevity, 2022:8059622 mój udział polegał na współtworzeniu konceptu oraz planu badawczego, nadzorze merytorycznym nad częścią laboratoryjną projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Ewa Barg, Paweł Reichert, Aleksandra Królikowska, 2022, „Effectiveness of Lateral Elbow Tendinopathy Treatment Depends on the Content of Biologically Active Compounds in Autologous Platelet-Rich Plasma", Journal of Clinical Medicine, 11(13):3687 mój udział polegał na przygotowaniu planu badawczego i metodologii badania, nadzorze merytorycznym nad częścią laboratoryjną projektu, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Wrocław, 22.08.2022

dr n. med. Jarosław Witkowski
Katedra Chirurgii Urazowej,
Wydział Lekarski,
Wrocławski Uniwersytet Medyczny
ul. Borowska 213, 50-556 Wrocław

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej, interpretacja zebranych danych, wprowadzaniem poprawek merytorycznych do pierwotnej wersji manuskryptu.

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na współudziale w przygotowaniu i zabezpieczeniu materiału biologicznego do dalszej analizy laboratoryjnej, współudziale w przygotowaniu bazy danych do dalszej analizy statystycznej, interpretacja zebranych danych.

Wrocław, 22.08.2022

dr hab. Piotr Morasiewicz
Instytut Nauk Medycznych
Wydział Lekarski,
Uniwersytet Opolski
al. Witosa 26, 45-401 Opole

OŚWIADCZENIE

Oświadczam, że w pracy:

Maciej Dejnek, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Ewa Barg, Jarosław Witkowski, Paweł Reichert, 2022, „Analysis and comparison of autologous platelet-rich plasma preparation systems used in the treatment of enthesopathies: A preliminary study", Advances in Clinical and Experimental Medicine, 30(7):757–764 mój udział polegał na współtworzeniu konceptu oraz planu badawczego, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

Maciej Dejnek, Jarosław Witkowski, Helena Moreira, Sylwia Płaczkowska, Piotr Morasiewicz, Paweł Reichert, Aleksandra Królikowska, 2022, „Content of blood cell components, inflammatory cytokines and growth factors in autologous platelet-rich plasma obtained by various methods", World Journal of Orthopedics, 13(6): 587-602 mój udział polegał na interpretacji uzyskanych wyników, krytycznej analizie pierwotnej wersji manuskryptu oraz wprowadzaniem do niej poprawek merytorycznych i językowych.

2. ŹRÓDŁA FINANSOWANIA

Prezentowane wyniki badań zrealizowano z pomocą subwencji Ministerstwa Zdrowia w kwocie 25 000,00zł w ramach tematu badawczego pt. „Ocena skuteczności leczenia entezopatii nadkłykcia bocznego kości ramiennej w zależności od zawartości składników biologicznie aktywnych w autologicznym osoczu bogatopłytkowym" ujętego w systemie Simple Wrocławskiego Uniwersytetu Medycznego pod nr STM.E067.20.112.

Niniejsze publikacje powstały w wyniku realizacji projektu pn. „Uniwersytet Medyczny we Wrocławiu jako Regionalny Ośrodek Doskonałości w dziedzinie nauk medycznych i nauk o zdrowiu" realizowanego w ramach środków Ministerstwa Nauki i Szkolnictwa Wyższego w Programie „Regionalna Inicjatywa Doskonałości" w latach 2019-2022, nr projektu 016/RID/2018/19, kwota 11 998 121,30zł. Decyzją Prorektora ds. Nauki UMW w dniu 10.07.2020 przyznano środki finansowe w wysokości 48 500 zł na realizację projektu badawczego w grupie wybitnych doktorantów pt. „Evaluation of clinical efficacy of different injection therapies for treating humeral epicondylopathies" (nr grantu RID.Z501.20.008).

3. ZGODY KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 26/2019

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

dr hab. Jacek Daroszewski, prof. nadzw. (endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Müller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
dr hab. Leszek Szenborn, prof. nadzw (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Ocena skuteczności leczenia entezopatii nadkłykcia bocznego i przyśrodkowego kości ramiennej"

zgłoszonym przez **lek. Macieja Dejneka** uczestnika studiów doktoranckich w Zakładzie Medycyny Sportowej Katedry Fizjoterapii Wydziału Nauk o Zdrowiu Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła wyrazić zgodę na przeprowadzenie badania w Zakładzie Medycyny Sportowej Katedry Fizjoterapii Wydziału Nauk o Zdrowiu Uniwersytetu Medycznego we Wrocławiu oraz w eMKa MED. Centrum Medycznym Bis s.c we Wrocławiu pod nadzorem dr hab. Pawła Reicherta, prof. nadzw. **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności:

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektu badawczego będącego podstawą rozprawy doktorskiej

Wrocław, dnia 21 stycznia 2019 r.

BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 163/2020

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Müller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn, (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Porównanie zawartości składników morfologicznych oraz czynników wzrostu w autologicznym osoczu bogatopłytkowym uzyskanym z wykorzystaniem różnych metod separacji grawitacyjnej stosowanych w ortobiologii"

zgłoszonym przez **lek. Macieja Dejneka** uczestnika studiów doktoranckich w Zakładzie Medycyny Sportowej Katedry Fizjoterapii Wydziału Nauk o Zdrowiu Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła wyrazić zgodę na przeprowadzenie badania w: Zakładzie Medycyny Sportowej Katedry Fizjoterapii Wydziału Nauk o Zdrowiu oraz w Diagnostycznym Laboratorium Naukowo-Dydaktycznym Katedry Diagnostyki Laboratoryjnej Uniwersytetu Medycznego we Wrocławiu pod nadzorem dr. hab. Pawła Reicherta **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności:

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektu badawczego będącego podstawą rozprawy doktorskiej

Wrocław, dnia 30 marca 2020 r.

BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel