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New biomarkers of the severity of acute pancreatitis.

Nowe biowskaźniki ciężkości przebiegu ostrego zapalenia trzustki.

Praca doktorska

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Spis treści

Nota informacyjna

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1. **Kolber W**., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int. J. Med. Sci.* 2018; 19: 1820, 10.3390/ijms19061820, (IF 3,687; MNiSW 30 punktów);

2. **Kolber W**., Kuśnierz-Cabala B., Dumnicka P., Maraj M., Mazur-Laskowska M., Pędziwiatr M., Ceranowicz P.: Serum urokinase-type plasminogen activator receptor does not outperform C-reactive protein and procalcitonin as an early marker of severity of acute pancreatitis. *J. Clin. Med.* 2018; 7: 305, doi: 10.3390/jcm7100305, (IF 5,583; MNiSW 15 punktów);

3. **Kolber W**., Kuśnierz-Cabala B., Maraj M., Kielar M., Mazur P., Maziarz B., Dumnicka P.: Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen receptor and interleukin 6 and predicts organ failure. *Folia Med. Cracov*. 2018; 4: 57-74, doi:10.24425, (MNiSW 10 punktów).

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Podsumowanie pracy doktorskiej w języku polskim

Wstęp

Ostre zapalenie trzustki (OZT) należy do stosunkowo często występujących ostrych schorzeń przewodu pokarmowego, które u blisko 80% pacjentów przybiera postać łagodną (*mild acute pancreatitis* - MAP), mijającą bez powikłań w ciągu kolejnych dni trwania choroby. Jednak, u ok. 15-25% chorych może dochodzić do rozwoju ciężkiej postaci SAP (*severe acute pancreatitis*), obarczonej blisko 20-30% śmiertelnością, która znacząco wzrasta w przypadku osób starszych oraz obciążonych dodatkowymi schorzeniami tj. przewlekła choroba nerek, choroby układu sercowo-naczyniowego, choroby autoimmunologiczne, czy cukrzyca [1-5]. Zaobserwowano dwa piki śmiertelności w przebiegu ostrego zapalenia trzustki, przy czym blisko 50% zgonów obserwowana jest w okresie pierwszego tygodnia trwania choroby i jest skorelowana z nasiloną uogólnioną odpowiedzią zapalną (SIRS) organizmu prowadzącą do rozwoju niewydolności narządowej [4].

W ocenie postępu medycyny jaki dokonał się w ciągu ostatnich lat w zakresie badań obrazowych (tomografia komputerowa z kontrastem, rezonans magnetyczny, ultrasonografia), uwagę zwraca zwiększenie dostępności do tych badań w chwili obecnej, jak również poprawa procesu diagnostycznego, tak w zakresie rozpoznawania OZT, jak również prognozowania rozwijających się wcześnie zmian martwiczych [1,3]. Zgodnie z aktualnie obowiązującymi zmodyfikowanymi kryteriami Atlanta 2012 [3], zmiany obserwowane w badaniach obrazowych pozostają obok typowego obrazu klinicznego i ponad 3-krotnego wzrostu aktywności enzymów trzustkowych (amylazy lub lipazy), jednym z kryteriów rozpoznania OZT. Ostre zapalenie trzustki rozpoznajemy, gdy obecne są przynajmniej dwa spośród trzech kryteriów [3,6].

Kryteria Atlanta 2012 [3] zdefiniowały również trzy stopnie ciężkości w przebiegu ostrego zapalenia trzustki, postać łagodną (MAP), średnio-ciężką (*moderately-severe acute pancreatitis* - MSAP) i ciężką (SAP) oraz dwa typy ostrego zapalenia trzustki: obrzękowe i martwicze ostre zapalenie trzustki [3]. Łagodną postać rozpoznaje się u pacjentów w przypadku braku niewydolności narządowej, powikłań ogólnoustrojowych oraz miejscowych. Postać umiarkowanie ciężką (MSAP) diagnozujemy u chorych, u których wystąpiła przemijająca niewydolność narządowa (ustępująca przed upływem 48 godzin), powikłania miejscowe tj. ostry okołotrzustkowy zbiornik płynowy, torbiel rzekoma, ostry zbiornik martwiczy, odizolowana martwica) lub ogólnoustrojowe (zaostrzenie

współwystępujących chorób przewlekłych). Ciężką postać schorzenia rozpoznajemy w przypadku obecności przetrwałej niewydolności narządowej (utrzymującej się powyżej 48 godzin) [3].

W ciągu ostatnich lat dokonał się również zasadniczy postęp w rozumieniu patomechanizmu ostrego zapalenia trzustki, a co za tym idzie poszerzeniu uległ panel badań diagnostycznych wykorzystywanych w celu wczesnego przewidywania SAP. Z kolei, ewaluacja dostępnych badań laboratoryjnych przyczyniła się do szerszego wdrożenia zarówno znanych od lat 70-80 tych XX wieku skal prognostycznych kliniczno-laboratoryjnych tj. skala Ransona, Glasgow, APACHE II (*Acute Physiology and Chronic Health Evaluation*), czy wdrożonej w ostatnim czasie BISAP score (*Bedside Index of Severity in AP*), ale również pozwoliła wyłonić pojedyncze markery, które uznano za pomocne w przewidywaniu SAP, np. białko C-reaktywne, azot mocznika (BUN), hematokryt [1].

Obserwacja stanu klinicznego pacjentów z OZT prowadzona przez lata oraz wytyczne zmodyfikowanej klasyfikacji Atlanta 2012 [3] wskazują, że bezwzględne „okno terapeutyczne", czyli czas, w którym podejmowane działania diagnostyczno-lecznicze mogą wpłynąć na poprawę stanu pacjentów, w tym obniżenie śmiertelności chorych, określane jest na okres pierwszych 48 godzin trwania ostrego zapalenia trzustki [3].

Do najpowszechniej stosowanych w rutynowej diagnostyce laboratoryjnych wskaźników prognostycznych wykorzystywanych u chorych z OZT w tym okresie należą przede wszystkim mediatory i markery stanu zapalnego tj. białka ostrej fazy (białko C-reaktywne, albumina, fibrynogen), prokalcytonina, wskaźnik prealbumina/fibrynogen, a także wapń całkowity, wskaźniki oceny funkcji nerek (mocznik, kreatynina), markery wątrobowe (aminotransferazy AST i ALT, fosfataza alkaliczna, bilirubina, dehydrogenaza mleczanowa), parametry krzepnięcia (D-dimery), całkowita liczba krwinek białych wraz z oceną liczebności poszczególnych populacji, a w ostatnim czasie również cytokiny prozapalne, przede wszystkim interleukina 6 (IL-6) [1,4-5].

Interleukina 6 jest glikoproteiną o masie cząsteczkowej 23 kDa produkowaną przez szereg komórek tj. monocyty, makrofagi, neutrofile, limfocyty T i B, komórki śródbłonka oraz fibroblasty w odpowiedzi na stymulację przez czynniki prozapalne tj. IL-1β oraz czynnik martwicy nowotworów (TNF-α) [4]. Większość najnowszych badań potwierdza znaczącą rolę IL-6 jako markera prognostycznego rozległości procesu zapalnego, w tym również we wczesnym prognozowaniu ciężkości przebiegu OZT, a także rozwoju niewydolności narządowej. Interleukina 6 wykazuje znaczącą dynamikę zmian stężenia we wczesnej fazie rozwoju OZT, a do jej uchwycenia wymagane jest wdrożenie technik pomiarowych

pozwalających na analizę zmian jej stężenia w czasie rzeczywistym.

Dotychczasowe badania prowadzone przez zespół badawczy, z którym współpracował Autor zwracają uwagę na istotne znaczenie markerów oceniających dysfunkcję śródbłonka naczyń krwionośnych tj. angiopoetyna-2 (Ang-2) i sFlt-1 (*soluble fms-like tyrosine kinase -1*) oraz zaburzeń krzepnięcia (D-dimery) [7-10]. Dla rozpoznawania ostrego uszkodzenia nerek (*AKI – acute kidney injury*) we wczesnej fazie trwania OZT istotne praktyczne znaczenie może mieć oznaczanie NGAL (*neutrophil gelatinase-associated lipocalin*) w moczu [10-11].

Zapalenie śródbłonka, łącznie z zaburzeniem perfuzji tkanek prowadzi do stopniowego rozwoju niewydolności narządowej będącej główną przyczyną zgonów we wczesnej fazie ostrego zapalenia trzustki [12-13]. Jednym z najwcześniejszych mediatorów fibrynolizy jest aktywator plazminogenu typu urokinazowego (uPA) [13]. Aktywacja uPA odbywa się poprzez wiązanie do jego receptora (uPAR, CD87), który ulega ekspresji na śródbłonku, a także aktywuje komórki T, granulocyty, monocyty i makrofagi zaangażowane w lokalną proteolizę i fibrynolizę [13-15]. Ponadto, uPAR bierze udział w adhezji komórek, migracji, chemotaksji, aktywacji immunologicznej, remodelingu tkanek oraz transdukcji sygnału [16]. Badania Wu et al. [13] u chorych leczonych w oddziałach intensywnego nadzoru potwierdziły dodatkowo istnienie dodatniej korelacji pomiędzy wzrostem osoczowego poziomu uPAR i ciężkością choroby ocenianą wg. skal APACHE II oraz SAPS II (*Sequential Organ Failure Assessment*) [13-15]. Z kolei badania Koch et al. [14] oraz Yu et al. [17] wskazują na zależność pomiędzy wzrostem stężenia uPAR oraz cytokinami prozapalnymi (IL-6, TNF-α), a także prokalcytoniną i białkiem C-reaktywnym [14, 17].

Cele pracy

Celem pracy było poszukiwanie nowych biomarkerów ostrego zapalenia trzustki, w szczególności:

1. Analiza zmian stężenia receptora aktywatora plazminogenu typu urokinazowego (uPAR) oraz interleukiny 6 (IL-6) w surowicy we wczesnej fazie przebiegu ostrego zapalenia trzustki oraz porównanie wartości diagnostycznej oznaczeń uPAR z wykorzystywanymi w bieżącej praktyce klinicznej pomiarami prokalcytoniny oraz białka C-reaktywnego;

2. Poszukiwanie korelacji pomiędzy zmianą stężeń uPAR i IL-6 w surowicy a kliniczną oceną ciężkości przebiegu ostrego zapalenia trzustki we wczesnej fazie choroby;

3. Ocena, w jakim stopniu wyniki wskazanych markerów, łącznie z danymi uzyskanymi podczas bieżącej, rutynowej diagnostyki chorych mogą mieć wpływ na zmianę algorytmu postępowania terapeutycznego w ostrym zapaleniu trzustki w celu ograniczenia liczby wczesnych powikłań zagrażających życiu.

Materiał i metodyka badań

Grupa badana

Do badania włączono pacjentów z rozpoznaniem ostrego zapalenia trzustki, hospitalizowanych i leczonych w Oddziale Chirurgicznym Zespołu Zakładów Opieki Zdrowotnej w Wadowicach. Do badania włączono pacjentów przyjmowanych w okresie pierwszych 24 godzin od wystąpienia objawów choroby.

Rozpoznanie ostrego zapalenia trzustki prowadzono w oparciu o zmodyfikowane kryteria Atlanta 2012 [3] zakładające obecność dwóch spośród 3 kryteriów zamieszczonych poniżej:

- obecność ostrego bólu w nadbrzuszu,
- aktywność enzymów trzustkowych (amylazy lub lipazy) w surowicy przekraczająca 3-krotnie wartość górnej granicy przedziału referencyjnego,

- charakterystyczne dla OZT zmiany widoczne w badaniach obrazowych (tomografia komputerowa z kontrastem, tomografia rezonansu magnetycznego lub badanie USG jamy brzusznej).

Kryteria wykluczenia

Do badania nie włączono pacjentów w przypadku:

- braku zgody pacjenta na udział w badaniu potwierdzonej własnoręcznym podpisem;
- obecności w chwili rozpoznania innych chorób tj. przewlekłe zapalenie trzustki, aktywna choroba nowotworowa, przewlekłe choroby wątroby (wirusowe zapalenie wątroby, marskość wątroby) oraz zdiagnozowana przewlekła choroba nerek,
- występowanie objawów (dolegliwości bólowych) powyżej 24 godzin do czasu zgłoszenia.

Charakterystyka grupy badanej

Do badania włączono łącznie 95 chorych z rozpoznaniem ostrego zapalenia trzustki, w tym 30 (32%) kobiet oraz 65 (68%) mężczyzn w wieku średnim 48±16,5 lat. W oparciu o klasyfikację Atlanta 2012 [3], u 29 (30,5%) chorych zdiagnozowano łagodną postać ostrego zapalenia trzustki (MAP), w przypadku 58 (61%) chorych postać średnio-ciężką ostrego zapalenia trzustki (MSAP), natomiast u 8 (8,5%) postać ciężką ostrego zapalenia trzustki (SAP). W trakcie leczenia 7 (7%) pacjentów wymagało przeniesienia do oddziału intensywnego nadzoru. Łącznie w przebiegu badania zmarło 4 (4%) chorych, w tym 1 pacjent we wczesnej fazie OZT, natomiast 3 chorych w późnej fazie trwania choroby.

Protokół badania

Badanie przeprowadzono w oparciu o protokół, który posiadał pozytywną opinię Komisji Bioetycznej (Opinia 2014/02/06/1 z dnia 6 lutego 2014 roku). Badanie dotyczyło poszukiwania optymalnego biowskaźnika użytecznego w przewidywaniu ciężkości przebiegu ostrego zapalenia trzustki we wczesnej fazie rozwoju choroby. U wszystkich chorych włączonych do badania czas trwania objawów przed przyjęciem na oddział nie przekraczał 24 godzin. W ramach badania, u każdego z pacjentów trzykrotnie pobierano próbki krwi oraz moczu: po upływie 24, 48 oraz 72 godzin od wystąpienia objawów ostrego zapalenia trzustki. Materiał pobierany był w celu przeprowadzenia niezbędnej diagnostyki i monitorowania stanu pacjenta, natomiast pozostałą po oznaczeniach surowicę oraz próbkę moczu zabezpieczano, porcjowano i mrożono w celu wykonania dodatkowych zaplanowanych oznaczeń tj. IL-6, uPAR oraz sFlt-1 (*soluble fms-like tyrosine kinase -1*). W próbkach moczu,

po wykonaniu badań ogólnych przeprowadzono oznaczenia: cząsteczki uszkodzenia nerek 1 (*kidney injury molekule-1, KIM-1*) oraz wątrobowego typu białka wiążącego kwasy tłuszczowe (*liver-type fatty acid binding protein, L-FABP*).

W toku badania zebrano i poddano analizie statystycznej dane kliniczne oraz wyniki badań laboratoryjnych. Oceniano wiek i płeć pacjentów, dane na temat chorób towarzyszących, przeprowadzono ocenę funkcji nerek, wątroby, układu sercowo-naczyniowego, płuc, przeanalizowano dane na temat wdrożonego leczenia (antybiotykoterapia, leczenie chirurgiczne), a także długości czasu hospitalizacji oraz wyników badań obrazowych. Do przewidywania ciężkości przebiegu ostrego zapalenia trzustki wykorzystywano dostępne wieloczynnikowe skale prognostyczne tj. skala BISAP, skala Ransona, skala BALI, PANC3 oraz Harmless acute pancreatitis score. Powikłania tj. ostre uszkodzenie nerek (*acute kidney injury, AKI*) diagnozowano w oparciu o kryteria KDIGO (*Kidney Disease: Improving Global Outcomes*) [18], natomiast niewydolność narządową w oparciu o zmodyfikowaną skalę Marshalla [1].

Badania laboratoryjne

W przeprowadzonych badaniach laboratoryjnych należy wyróżnić dwa kierunki: badania zlecane w trybie rutynowym, które wykonywane były podczas hospitalizacji w Centralnym Laboratorium Zespołu Zakładów Opieki Zdrowotnej w Wadowicach w celach diagnostycznych tj. badanie morfologii krwi obwodowej z rozmazem, badania biochemiczne w surowicy i w moczu oraz badania koagulologiczne. W diagnostyce tej wykorzystano rutynowo dostępne metody diagnostyczne oraz aparaturę pozostającą na wyposażeniu laboratorium tj. automatyczny analizator hematologiczny Sysmex XN (Sysmex Corporation, Cobe, Japonia), analizatory Cobas E411 (Roche Diagnostics, Mannheim, Niemcy) i Vitros 5600 (Ortho Clinical Diagnostics, Raritan, NJ, USA) do badań biochemicznych i immunochemicznych, natomiast badania układu krzepnięcia wykonano na aparacie Coag XL (Diagon, Budapeszt, Węgry). Dodatkowe badania laboratoryjne (IL-6 oraz sFlt-1) oznaczono w zabezpieczonym materiale, metodą elektrochemiluminescencyjną na analizatorze Cobas 8000 (Roche Diagnostics, Mannheim, Niemcy) w Zakładzie Diagnostyki Szpitala Uniwersyteckiego w Krakowie, natomiast oznaczenia stężenia uPAR w surowicy (Human Quantikine uPAR Immunoassay R&D Systems, Minneapolis, MN, USA), KIM-1 w moczu (Human Quantikine TIM-1/KIM-1/HAVCR Immunoassay R&D Systems, Minneapolis, MN, USA) oraz L-FABP w moczu (CMIC Holding Co., Tokyo, Japonia)

wykonano metodami immunoenzymatycznymi w Zakładzie Diagnostyki Katedry Biochemii Klinicznej UJ CM w Krakowie.
Zakresy wartości referencyjnych dla wykonywanych badań laboratoryjnych przedstawiono w *Załączniku nr 1*.

Analiza statystyczna

Dane jakościowe przedstawiono jako liczbę pacjentów (odsetek w grupie badanej). W przypadku rozkładu zmiennych ilościowych zastosowano test Shapiro-Wilka, natomiast dane ilościowe przedstawiano jako wartość średnią wraz z odchyleniem standardowym lub jako medianę (dolny-górny kwartyl), w zależności od typu rozkładu danych (normalnego lub różnego od normalnego). W ocenie liczebności stosowano test chi-kwadrat, natomiast do oceny różnic pomiędzy grupami wykorzystywano testy: t-Studenta, Manna-Whitney'a jednoczynnikowej analizy wariancji i Kruskala-Wallisa (w zależności od liczby grup i rozkładu zmiennych). Do oceny korelacji, w zależności od typu rozkładu danych wyliczano współczynniki korelacji R Persona lub Spearmana. W celu oceny, czy badane związki pozostają niezależne od zmiennych towarzyszących przeprowadzano analizę regresji wielokrotnej. W ocenie regresji liniowej wykorzystywano zmienne oryginalne. W celu oceny użyteczności diagnostycznej analizowanych parametrów wykorzystywano analizę krzywych ROC (*reciver operating characteristics*), wyliczano wartości czułości oraz swoistości diagnostycznej oraz wyznaczano optymalne punkty odcięcia. Wyniki analizy statystycznej przeprowadzono zakładając, że są istotne statystycznie dla $p \leq 0,05$. We wszystkich trzech pracach w analizie statystycznej do obliczeń wykorzystano oprogramowanie Statistica 12.0 (StatSoft, Tulsa, USA) wraz z Zestawem Medycznym (StatSoft Polska, Kraków, Polska).

Zakres tematyczny artykułów wchodzących w skład cyklu i główne wyniki

Artykuł nr 1

Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis. *Int. J. Mol. Sci.* 2018; 19: 1821; doi.10.3390/ijms19061820

W artykule tym podjęto próbę weryfikacji użyteczności diagnostycznej stężenia interleukiny 6 jako czynnika prognostycznego ciężkiego przebiegu OZT, rozwoju niewydolności narządowej oraz pojawienia się wskazań do wdrożenia intensywnej terapii i zagrożenia zgonem. Badanie przeprowadzono w grupie 95 dorosłych chorych, u których rozpoznano OZT zgodnie z kryteriami z Atlanty w modyfikacji z 2012 roku. U 29 chorych stwierdzono postać łagodną (MAP), u 58 postać średnio-ciężką (MSAP), a u 8 postać ciężką OZT (SAP). Stężenie IL-6 oceniono w chwili przyjęcia oraz w kolejnym dniu hospitalizacji. U pacjentów z SAP w chwili przyjęcia obserwowano znamiennie wyższe stężenia IL-6 w surowicy w porównaniu do postaci MAP i MSAP. Dodatkowo, w obu dniach badania, stężenie IL-6 było dodatnio skorelowane z długością hospitalizacji, punktacją w skali Ransona, a także z wczesnymi markerami ostrej niewydolności nerek (AKI) tj. KIM-1 i L-FABP oraz markerami dysfunkcji śródbłonka (Ang-2 oraz sFlt-1). Potwierdzono również obecność korelacji pomiędzy stężeniem IL-6 w surowicy oraz dobrze rozpoznawanymi w praktyce klinicznej markerami stanu zapalnego tj. całkowitą liczbą leukocytów oraz bezwzględną liczbą neutrofili, stężeniem białka C-reaktywnego oraz prokalcytoniny. Ważnym aspektem przeprowadzonych badań była zaproponowana metodyka oznaczeń IL-6 w surowicy z wykorzystaniem w pełni zautomatyzowanej platformy analitycznej umożliwiającej monitorowanie zmian stężenia IL-6 w trybie diagnostyki rutynowej.

Analiza dynamiki zmian stężenia IL-6 w surowicy prowadzona u chorych we wczesnej fazie rozwoju OZT wskazuje na jej znaczącą rolę jako czynnika prognostycznego wystąpienia ciężkiej postaci OZT, przetrwałej dysfunkcji narządowej (Marshall score ≥2), oraz czynnik prognostyczny konieczności wdrożenia intensywnej terapii i śmiertelności w tej grupie chorych. W opinii Autorów, zastosowanie w pełni automatycznych metod oznaczeń stężenia IL-6 w surowicy umożliwia szybkie i powtarzalne oznaczanie stężenia IL-6 pozwalające na wykorzystanie jej wyników w diagnostyce różnicowej.

Artykuł nr 2

Kolber W., Kuśnierz-Cabala B., Dumnicka P., Maraj M., Mazur-Laskowska M., Pędziwiatr M., Ceranowicz P.: Serum urokinase - type plasminogen activator receptor does not outperform C-reactive protein and procalcitonin as an early marker of severity of acute pancreatitis. J. Clin. Med. 2018; 7: 306; doi.10.3390/jcm7100305

Celem pracy było porównanie wartości diagnostycznej pojedynczych oznaczeń uPAR w surowicy z innymi wskaźnikami stanu zapalnego, jako czynników prognostycznych ciężkiego przebiegu OZT. Wykazano, że zmiany stężenia uPAR w surowicy w chwili przyjęcia pozwalają na prognozowanie SAP, niewydolności narządowej, głównie ostrego uszkodzenia nerek oraz niewydolności sercowo-naczyniowej, czy podjęcia decyzji o przeniesieniu pacjenta do oddziału ICU. Wykazano również istnienie korelacji pomiędzy stężeniami uPAR w surowicy oraz laboratoryjnymi markerami uszkodzenia wątroby tj. AST, ALT i bilirubiną. Po przeprowadzeniu analizy użyteczności diagnostycznej testu, oceniając wielkość pola pod krzywą ROC (AUC) dla uPAR, wykazano, że nie różni się ona znacząco od innych porównywanych z nim markerów tj. IL-6, CRP, PCT, D-dimery oraz sFlt-1. Do oznaczeń uPAR w surowicy wykorzystywano technikę pomiaru ELISA, a co za tym idzie, oznaczenie tego parametru nie posiada odpowiedniej standaryzacji laboratoryjnej. Pomimo obiecujących wstępnych wyników, autorzy formułują ostrożne wnioski końcowe w zakresie wdrożenia oznaczeń uPAR w celu prognozowania SAP. Badania wymagają potwierdzenia dotychczasowych obserwacji w grupie o większej liczebności, w szczególności z SAP. Na tę chwilę należy uznać, że oznaczanie stężenia uPAR w surowicy w celu prognozowania ciężkości przebiegu OZT we wczesnej fazie rozwoju choroby nie wnosi większych korzyści od stosowanych rutynowo i uznanych testów tj. CRP, PCT, czy D-dimery.

Artykuł nr 3

Kolber W., Kuśnierz-Cabala B., Maraj M., Kielar M., Mazur P., Maziarz B., Dumnicka P.: Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure. Folia Med Cracov. 2018; 4: 57-74; doi.10.24425/fmc.2018 125704.

W pracy tej przeprowadzono ocenę korelacji pomiędzy stężeniami IL-6 i uPAR w surowicy oraz wskaźnikami wyliczanymi w oparciu o bezwzględne liczby neutrofili do limfocytów

(NLR), limfocytów do monocytów (LMR) oraz płytek krwi do limfocytów (PLR) u chorych z OZT we wczesnej fazie trwania choroby. Wskaźniki wyliczano podczas wykonywanych rutynowo badań morfologii krwi z rozmazem w każdym z trzech dni badania. W oparciu o zmodyfikowaną skalę Marshalla (MMSS), u pacjentów z niewydolnością narządową (MMSS ≥2 punktów) wykazano znamiennie statystycznie niższą wartość wskaźnika LMR w pierwszej dobie trwania OZT. W drugim i trzecim dniu badania obserwowano znamiennie wyższe wartości wskaźnika NLR u pacjentów o cięższym przebiegu OZT. Analiza korelacji wykazała istnienie dodatniej korelacji pomiędzy wartością wskaźnika NLR oraz stężeniami IL-6, PCT oraz uPAR w każdym dniu badania. W każdym dniu badania obserwowano również ujemną korelację pomiędzy bezwzględną liczbą limfocytów oraz stężeniami IL-6, PCT oraz uPAR w surowicy krwi chorych. Dodatkowo, po upływie 48 godzin obserwacji potwierdzono obecność korelacji pomiędzy całkowitą liczbą leukocytów, bezwzględną liczbą neutrofili oraz wskaźnikiem NLR ze stopniem ciężkości chorych ocenianym wg. skali Ransona.

Ograniczenia badania

Do badania włączono łącznie 95 chorych z ostrym zapaleniem trzustki, przy czym grupa z ciężką postacią (SAP) liczyła jedynie 8 chorych. Jednym z czynników mogących tłumaczyć trudności w uzyskaniu odpowiednio liczebnej grupy chorych z SAP jest wstępna selekcja polegająca na kierowaniu tych chorych bezpośrednio do leczenia w ośrodkach referencyjnych. Wyciąganie wniosków klinicznych w oparciu o tak nieliczną grupę chorych z SAP, w szczególności w kontekście oceny ich użyteczności diagnostycznej nakazuje zachowanie ostrożności. Autorzy są świadomi tego ograniczenia i w przedstawionych pracach wchodzących w skład rozprawy formułują wnioski z zaznaczeniem konieczności zwiększenia liczebności grupy badanej (głównie z SAP) podczas prowadzenia dalszych badań w zakresie omawianej problematyki mającej na celu wdrożenie ocenianych biowskaźników w przyszłości do panelu szeroko stosowanych badań rutynowych.

Wnioski

1. U pacjentów z SAP wykazano znamiennie wyższe stężenia IL-6 zarówno w chwili rozpoznania OZT, jak również w drugim dniu badania w porównaniu do chorych z MAP oraz MSAP. W oparciu o analizę krzywych ROC, w chwili przyjęcia na oddział wyznaczono optymalny punkt odcięcia dla stężenia IL-6 w surowicy >211 pg/mL dla przewidywania SAP, IL-6 >262 pg/mL wskazujący na zwiększone ryzyko rozwoju powikłań narządowych oraz IL-6 >229 pg/mL przewidujący konieczność przeniesienia pacjenta do leczenia w oddziale intensywnego nadzoru i zwiększone ryzyko zgonu pacjenta.

2. Analiza zmian stężenia IL-6 w surowicy potwierdziła istnienie znamiennych korelacji jej stężenia z długością hospitalizacji chorych oraz ciężkością OZT ocenianą w oparciu o skalę Ransona, podobnie jak z wybranymi laboratoryjnymi wskaźnikami stanu zapalnego (całkowita liczba leukocytów, bezwzględna liczna neutrofili, stężenie albuminy, białka C-reaktywnego, prokalcytoniny), markerami uszkodzenia śródbłonka naczyń krwionośnych (sFlt-1, Ang-2), a także wybranymi wskaźnikami dysfunkcji narządowych (KIM-1, L-FABP, LDH, bilirubina).

3. W grupie z SAP obserwowano wyższe stężenia uPAR w porównaniu do pacjentów z MAP i MSAP we wszystkich dniach badania, przy czym różnice te były znamienne statystycznie jedynie w 2-dobie obserwacji. Na podstawie oceny wartości ilorazu szans wykazano, że w oparciu o pomiar uPAR w surowicy w pierwszym dniu badania możliwe jest przewidywanie wystąpienia niewydolności narządowej (MMSS≥2; OR=2,14), w szczególności rozwoju ostrej niewydolności krążeniowej (OR=2,33), ostrej niewydolności nerek (OR=1,78), a także konieczności przeniesienia pacjenta do leczenia w oddziale intensywnego nadzoru (OR=2,06) oraz zgonu pacjenta (OR=1,82).

4. Wykazano istnienie znamiennych statystycznie zależności pomiędzy stężeniami uPAR w surowicy oraz wybranymi markerami zapalnymi tj.; albuminą, prokalcytoniną i IL-6 w każdym dniu badania oraz CRP począwszy od drugiej doby badania. Zaobserwowano ponadto obecność dodatniej korelacji pomiędzy stężeniem uPAR w surowicy oraz wskaźnikiem dysfunkcji śródbłonka naczyń krwionośnych (sFlt-1

w pierwszej dobie), laboratoryjnymi markerami zaburzeń funkcji wątroby (AST, ALT i LDH w każdym dniu badania oraz bilirubiną począwszy od 2 doby), a także stężeniem D-dimerów (1 i 3 doba badania).

5. Wyliczone wartości pola powierzchni pod krzywą ROC (AUC) dla uPAR w dniu przyjęcia dla przewidywania SAP, niewydolności narządowej oraz konieczności leczenia w oddziale intensywnej terapii i zgonu wynoszą odpowiednio 0,641; 0,761 oraz 0,759, nie przewyższają wartości uzyskanych dla przewidywania SAP przez takie markery jak: IL-6 (AUC: 0,753), CRP (AUC: 0,647), PCT (AUC: 0,669) oraz D-dimery (AUC: 0,605).

Implikacje praktyczne pracy

Na podstawie dostępnego, bogatego piśmiennictwa wielokrotnie potwierdzono istotne znaczenie IL-6 w patomechanizmie zarówno rozwoju ostrego zapalenia trzustki, jak również jego powikłań, tak wczesnych, jak i późnych. Dostępne w większości opracowań techniki pomiaru dotyczą głównie metody ELISA. Znaczący rozwój technologii diagnostycznej jaki dokonuje się od kilku lat pozwala na zaadoptowanie do celów diagnostyki rutynowej wielu markerów dotychczas nie wykonywanych. Celem praktycznym przeprowadzonego badania była ocena użyteczności automatycznego pomiaru IL-6 w surowicy u chorych we wczesnym prognozowaniu SAP. Uzyskane i opublikowane w *Artykule nr 1* wyniki potwierdzają wysoką użyteczność diagnostyczną pomiarów IL-6 w diagnostyce ostrego zapalenia trzustki. Jednocześnie, szybkość uzyskiwania w pełni powtarzalnego wyniku badania stanowi ważne osiągnięcie w dotychczasowej diagnostyce w tej grupie schorzeń.

Pomimo wstępnych, obiecujących wyników wskazujących na użyteczność pomiarów uPAR w surowicy w prognozowaniu powikłanego przebiegu ostrego zapalenia trzustki, wartość diagnostyczna pomiarów nie przewyższa innych markerów stosowanych aktualnie w diagnostyce tj. białko C-reaktywne, prokalcytonina, czy D-dimery. Przeprowadzone badanie *(Artykuł 2)* dostarczyło tym samym istotnych praktycznych informacji na temat stosunkowo ograniczonej roli tego parametru we wczesnym prognozowaniu powikłań SAP, jednocześnie dokumentując, że nie spełnia on wszystkich cech oczekiwanych od wskaźnika, który chcemy uznać za biomarker.

Wykazane w *Artykule nr 3* istotne korelacje pomiędzy zmianą stężenia IL-6 i uPAR w surowicy oraz wartością wskaźników: neutrofilowo-limfocytarnego (NLR), limfocytarno-monocytarnego (LMR) oraz płytkowo-limfocytarnego (PLR) wskazują na możliwości ich praktycznego wykorzystania. Wyliczanie w/w wskaźników podczas prostego badania morfologii krwi z rozmazem kieruje uwagę na potencjał jaki posiadają dobrze znane, chociaż stosunkowo mało rozpowszechnione badania w codziennej praktyce klinicznej u chorych z ostrym zapaleniem trzustki.

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Artykuł nr 1

Witold Kolber, Paulina Dumnicka, Małgorzata Maraj, Beata Kuśnierz-Cabala, Piotr Ceranowicz, Michał Pędziwiatr, Barbara Maziarz, Małgorzata Mazur-Laskowska, Marek Kuźniewski, Mateusz Sporek, Jerzy Walocha

Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis

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Does the Automatic Measurement of Interleukin 6 Allow for Prediction of Complications during the First 48 h of Acute Pancreatitis?

Witold Kolber [1], Paulina Dumnicka [2], Małgorzata Maraj [3], Beata Kuśnierz-Cabala [4], Piotr Ceranowicz [3,*], Michał Pędziwiatr [5], Barbara Maziarz [4], Małgorzata Mazur-Laskowska [6], Marek Kuźniewski [7], Mateusz Sporek [8] and Jerzy Walocha [8]

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Abstract: Acute pancreatitis (AP) in most patients takes a course of self-limiting local inflammation. However, up to 20% of patients develop severe AP (SAP), associated with systemic inflammation and/or pancreatic necrosis. Early prediction of SAP allows for the appropriate intensive treatment of severe cases, which reduces mortality. Serum interleukin-6 (IL-6) has been proposed as a biomarker to assist early diagnosis of SAP, however, most data come from studies utilizing IL-6 measurements with ELISA. Our aim was to verify the diagnostic usefulness of IL-6 for the prediction of SAP, organ failure, and need for intensive care in the course of AP using a fully automated assay. The study included 95 adult patients with AP of various severity (29 mild, 58 moderately-severe, 8 severe) admitted to a hospital within 24 h from the onset of symptoms. Serum IL-6 was measured using electochemiluminescence immunoassay in samples collected on admission and on the next day of hospital stay. On both days, patients with SAP presented the highest IL-6 levels. IL-6 correlated positively with other inflammatory markers (white blood cell and neutrophil counts, C-reactive protein, procalcitonin), the markers of renal injury (kidney injury molecule-1 and neutrophil gelatinase-associated lipocalin), and the markers of endothelial dysfunction (angiopoietin-2, soluble fms-like tyrosine kinase-1). IL-6 on admission significantly predicted SAP, vital organ failure, and the need for intensive care or death, with areas under the receiver operating curve between 0.75 and 0.78, not significantly different from multi-variable prognostic scores. The fully automated assay allows for fast and repeatable measurements of serum IL-6, enabling wider clinical use of this valuable biomarker.

Keywords: interleukin 6; acute pancreatitis; severity; prediction of acute pancreatitis; organ failure

1. Introduction

Acute pancreatitis (AP) is a fairly common disease, relatively mild and self-limiting in most patients. However, about 15–20% of patients develop severe acute pancreatitis (SAP) associated with serious complications in both early and late phases of the disease, causing a mortality rate of 20–30% [1–3].

As there is no causative treatment, clinical studies and current recommendations point towards early diagnosis of SAP, transfer to intensive care unit (ICU), implementation of supportive fluid therapy, pain relief, control of intra-abdominal pressure, and early enteral feeding as the means to improve survival in severe cases [4,5].

Difficulty in establishing universal guidelines for the management of patients with SAP arises from its dynamic progression, and even patients admitted with apparently mild symptoms may progress to SAP. The loss of local control of the inflammatory site leads to systemic activation of neutrophils and monocytes, systemic inflammatory response syndrome (SIRS), followed by organ failure [6]. In some patients, death, due to cytokine storm, may in fact occur in the first 48 h of AP (fulminant AP). Persistent organ failure is the main cause of death in the early phase of SAP, while in the late phase, the infection of pancreatic necrosis may further aggravate prognosis (critical AP) [7,8].

A wide range of diagnostic tests is used for the evaluation of AP severity in early phase, including imaging tests (primarily computed tomography with contrast, or magnetic resonance imaging), multi-variable prognostic scales (e.g., bedside index of severity in AP (BISAP)), Ranson's scale, Glasgow scale, Acute Physiology and Chronic Health Evaluation (APACHE II), as well as single laboratory markers [9,10]. Among single biomarkers, C-reactive protein (CRP) and procalcitonin (PCT) are most widely used in daily clinical practice [11–13].

Since 1988, when Rinderknecht proposed a new pathophysiological concept to describe a complex network of inflammatory mediators released by activated leukocytes, clinical studies explored a wide range of inflammatory cytokines, chemokines, reactive oxygen species, adhesion molecules, and acute phase proteins as potential predictors of SAP [13–17]. In addition, the role of inflammatory cells and the mechanisms associated with the development of SIRS and compensatory anti-inflammatory response syndrome (CARS) in the course of SAP were extensively studied [18–21]. It is currently possible to measure the concentrations of both pro-inflammatory (tumor necrosis factor-α—TNFα, interleukins: IL-1β, IL-6, IL-8, IL-18, IL-33) and anti-inflammatory cytokines (IL-1 receptor antagonist—IL-1Ra, IL-10) [2,21–24]. Moreover, the studies by Huan et al. [25] and Manohar et al. [26] indicate that some cytokines, i.e., IL-6 and IL-22, can act as both pro- and anti-inflammatory agents [20].

Interleukin 6 is a glycoprotein with a mass of 26 kDa produced by numerous cells: monocytes, T-cells, B-cells, neutrophils, fibroblasts, and pancreatic acinar cells [2]. It has a pleiotropic effect and in acute inflammation, it restricts the synthesis of TNF-α and IL-1β with a concomitant increase in IL-1Ra synthesis and the release of the soluble TNF-α-receptor [27]. IL-6 stimulates the differentiation of antibody producing cells, macrophages, and Th17-cells [20], and induces the production of C-reactive protein from hepatocytes [1,14,15]. Interleukin 6 also acts as an anti-inflammatory mediator by stimulating IL-10 secretion [20]. Moreover, naive Th17 cells are generated in the presence of IL-6 and differentiated Th17 cells proliferate under the influence of IL-23, in turn inducting IL-23R mRNA expression [20]. In experimental AP in rats, an increase in serum IL-6 levels precede severe pancreatic edema and necrosis [28]. In humans, IL-6 is an earlier marker of severity in AP than C-reactive protein, most commonly used in clinical practice [14]. Notably, a recent study of Jain et al. [29] showed that the assessment of serum IL-6 increases the accuracy of SIRS to predict severe AP.

For a long time, measurements of serum IL-6 concentrations were not routinely available, as enzyme-linked immunosorbent assays (ELISA) did not allow for appropriately short turn-around times. In the last 20 years, significant advances in laboratory techniques enabled the development and expansion of automated assays to measure IL-6 concentrations, i.e., immunochemiluminometric assay (ECLIA), available on the Cobas Roche platform.

The authors analyzed serum IL-6 concentrations during the first 48 h from the onset of AP among patients with various degrees of AP severity. The aim of the study was to assess whether serum IL-6, measured via automatized laboratory assay, may serve as the early indicator of SAP and support a decision to urgently transfer a patient with predicted SAP to the ICU.

2. Results

The study included 95 patients with AP. The mean age of the studied AP patients was nearly 50 years and most patients were male (Table 1). Biliary and alcoholic etiologies of AP were almost equally common, however, one-third of patients had unknown etiology. Comorbidities were present in 44% of studied group, liver and cardiac diseases being the most prevalent. Based on 2012 Atlanta classification criteria, most patients (61%) were diagnosed with moderately severe AP (MSAP), mild AP (MAP) was diagnosed in 30% and severe AP (SAP) in 8% (Table 1). Pancreatic necrosis was observed in 13% of patients. Consequently, 75% of patients required more than one-week hospital stay. Intensive care was implemented in 7% and mortality was 4% (Table 1).

Table 1. Clinical characteristics of the study group of 95 patients with AP.

Characteristics	Observed Values
Mean age ± SD, years	48 ± 16.5
Males, *n* (%)	65 (68)
Severity of AP	
MAP, *n* (%)	29 (30.5)
MSAP, *n* (%)	58 (61)
SAP, *n* (%)	8 (8.5)
Etiology	
Biliary, *n* (%)	27 (28)
Alcoholic, *n* (%)	29 (31)
Alcohol plus high-fat diet, *n* (%)	12 (13)
Idiopathic, *n* (%)	20 (21)
Hipertriglicerydemia, *n* (%)	5 (5)
Other, *n* (%)	2 (2)
Median duration of hospital stay (Q1–Q3), days	12 (8–15)
SIRS in first 24 h, *n* (%)	74 (78)
Necrosis, *n* (%)	12 (13)
Early/late mortality, *n* (%)	1 (1)/3 (3)
BISAP score ≥ 3 in first 24 h; *n* (%)	21 (22)
Ranson score ≥ 3 in first 48 h; *n* (%)	29 (30.5)
BALI scale during first 48 h ≥ 3 points; *n* (%)	4 (4)
PANC3 score positive in first 24 h, *n* (%)	9 (9)
Harmless AP (according to HAPS), *n* (%)	46 (48)
Pre-existing comorbidities, *n* (%)	42 (44)
Cardiac diseases, *n* (%)	31 (33)
Liver disease, *n* (%)	32 (34)
Diabetes, *n* (%)	8 (8)
Dyslipidemia, *n* (%)	3 (3)
Chronic kidney disease, *n* (%)	2 (2)
Other comorbidities, *n* (%)	4 (4)
Antibiotic treatment, *n* (%)	87 (91.5)
Therapeutic ERCP, *n* (%)	5 (5)
Surgery, *n* (%)	8 (8)
Enteral feeding via nasojejunal tube, *n* (%)	10 (10.5)
Parenteral feeding, *n* (%)	3 (3)
Transfer to intensive care unit, *n* (%)	7 (7)

AP—acute pancreatitis; *n*—number of patients; MAP—mild acute pancreatitis; MSAP—moderately severe acute pancreatitis; SAP—severe acute pancreatitis; SD—standard deviation; Q1—lower quartile; Q3—upper quartile; SIRS—systemic inflammatory response syndrome; BISAP—bedside index of severity in AP; HAPS—harmless acute pancreatitis score; ERCP—endoscopic retrograde cholangiopancreatography.

Laboratory tests, including serum concentrations of IL-6 were repeated twice: on admission (i.e., study day 1) and on the second day of hospital stay (day 2) (Table 2). No significant changes in IL-6 concentrations were observed between measurements on admission and on second day of hospital stay, regardless of AP severity (Table 2). All studied patients were admitted within 24 h from the onset of symptoms, with a subgroup of 43 patients (45%) admitted within the first 12 h. The severity of AP was comparable between patients admitted within the first 12 h and those admitted later (p = 0.7). We did not observe significant differences between those subgroups regarding IL-6 concentrations on admission (p = 0.9) and on second day of hospital stay (p = 0.2).

Table 2. The results of laboratory tests on admission (day 1) and on day 2 of hospital stay according to severity of AP.

Variables	Study Day	Median (Q1-Q3)			
		MAP (n = 29)	MSAP (n = 58)	SAP (n = 8)	p
Interleukin 6, pg/mL	1	64.7 (14.8–95.7)	78.9 (27.8–163.0)	210.7 (73.1–2145.0)	0.037 [a]
	2	38.7 (9.2–103.3)	66.9 (33.6–219.5)	280.2 (98.9–528.2)	0.004 [a,b]
sFlt-1, pg/mL	1	129 (119–169)	140 (112–154)	191 (155–536)	0.1
	2	113 (108–145)	129 (101–161)	156 (146–209)	0.2
Ang-2, ng/mL	1	2.96 (2.03–3.64)	3.19 (2.39–3.72)	8.68 (5.11–18.8)	0.1
	2	3.47 (2.96–6.69)	3.09 (2.55–4.05)	9.02 (5.34–19.8)	0.041 [b]
CRP, mg/L	1	22.7 (5.30–132.4)	25.4 (11.9–174.7)	129.6 (17.4–316.7)	0.4
	2	164.7 (40.4–313.6)	268.6 (97.0–371.3)	384.8 (334.7–415.2)	0.015 [a]
Albumin, g/L	1	41.0 (31.0–44.0)	35.0 (33.0–37.0)	37.0 (35.0–39.0)	0.6
	2	36.0 (30.0–38.0)	32.0 (29.0–35.0)	24.0 (20.0–34.0)	0.033 [a]
PCT, ng/mL	1	0.10 (0.05–0.55)	0.17 (0.10–0.36)	0.61 (0.14–1.03)	0.1
	2	0.22 (0.05–0.59)	0.46 (0.14–1.43)	1.76 (0.84–5.29)	0.009 [a]
Total protein, g/L	1	78.0 (65.0–80.0)	65.0 (61.0–74.0)	76.5 (76.0–77.0)	0.017 [c]
	2	66.7 (64.0–69.0)	60.0 (53.0–64.0)	62.0 (59.0–66.0)	0.012 [c]
Hematocrit, %	1	42.4 (38.1–45.7)	43.8 (40.6–46.6)	44.5 (42.3–49.4)	0.4
	2	37.6 (36.5–39.8)	38.1 (36.3–41.5)	41.7 (37.1–45.7)	0.3
WBC, $\times 10^3/\mu L$	1	12.4 (9.5–15.2)	13.1 (10.4–16.2)	17.1 (10.3–23.3)	0.5
	2	8.6 (6.6–11.1)	10.4 (7.5–13.4)	13.7 (8.19–17.0)	0.054
NEU, $\times 10^3/\mu L$	1	11.8 (6.9–14.9)	10.5 (7.5–14.0)	9.2 (4.7–30.2)	0.9
	2	5.9 (4.3–8.1)	8.1 (5.7–11.3)	10.6 (5.6–13.1)	0.056
Bilirubin, µmol/L	1	23.4 (13.5–38.5)	27.2 (13.8–53.3)	29.1 (16.2–36.9)	0.8
	2	18.3 (13.2–27.0)	18.9 (13.7–28.2)	40.3 (39.6–43.3)	0.1
LDH, U/L	1	553 (488–810)	636 (507–850)	1012 (736–1293)	0.1
	2	526 (448–719)	603 (483–886)	1955 (1033–3058)	0.037 [a]
Total calcium, mmol/L	1	2.11 (1.90–2.37)	2.13 (2.04–2.24)	1.95 (1.87–2.27)	0.5
	2	2.05 (2.00–2.16)	2.11 (2.01–2.19)	1.90 (1.45–2.02)	0.020 [b]
Urea, mmol/L	1	3.67 (2.83–6.00)	4.67 (3.50–6.00)	6.67 (5.00–13.0)	0.020 [a]
	2	3.17 (2.83–4.00)	4.67 (3.17–6.17)	15.0 (8.17–18.50)	<0.001 [a,b,c]
Creatinine, µmol/L	1	65.4 (59.2–80.4)	69.8 (60.1–87.5)	92.4 (75.6–171.0)	0.036 [a]
	2	61.0 (46.9–68.1)	60.1 (51.3–71.6)	123.3 (68.5–204.6)	0.014 [a,b]
KIM-1, ng/mL	1	2.73 (1.30–5.11)	3.41 (2.07–6.14)	2.59 (1.63–4.74)	0.6
	2	2.15 (1.31–3.96)	2.75 (1.66–5.53)	1.82 (1.58–4.01)	0.2
L-FABP, ng/mL	1	5.82 (4.59–13.97)	22.52 (8.18–53.06)	12.84 (6.78–18.90)	0.2
	2	4.51 (3.84–7.56)	17.36 (4.01–30.34)	10.18 (8.03–12.33)	0.3

Ang-2—angiopoietin 2; CRP—C-reactive protein; KIM-1—kidney injury molecule-1; LDH—lactate dehydrogenase; L-FABP—liver-type fatty acid binding protein; MAP—mild acute pancreatitis; MSAP—moderately severe acute pancreatitis; NEU—neutrophils; PCT—procalcitonin; SAP—severe acute pancreatitis; sFlt-1—soluble fms-like tyrosine kinase -1; WBC—white blood cells; [a] significant difference between MAP and SAP groups in post-hoc comparison; [b] significant difference between MSAP and SAP groups in post-hoc comparison; [c] significant difference between MAP and MSAP groups in post-hoc comparison.

Serum concentrations of IL-6 did not differ significantly between patients with various etiologies of AP, although lowest IL-6 levels were observed in AP due to hypertriglyceridemia (Figure 1).

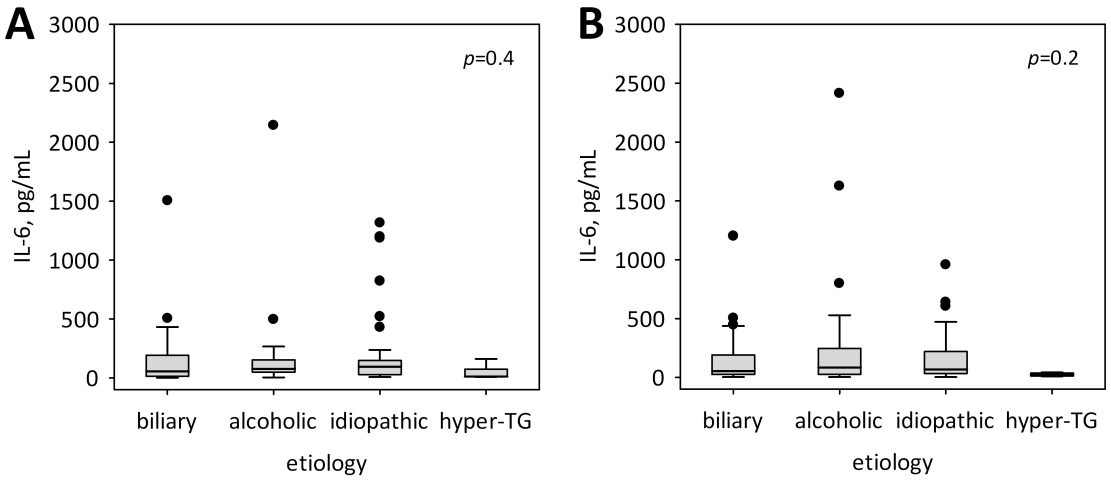

Figure 1. Serum concentrations of IL-6 among patients with various etiology of acute pancreatitis (AP) at admission (**A**) and on day 2 of hospital stay (**B**). Data are shown as median, interquartile range (box), non-outlier range (whiskers) and outliers (points).

Patients with SAP had highest serum IL-6 concentrations on both days of the study (Table 2). Moreover, higher IL-6 concentrations were observed among patients who subsequently developed necrotizing pancreatitis (Figure 2), however, significant difference was achieved on day 2 of hospital stay. Both on admission and on study day 2, IL-6 concentrations were positively correlated with the length of hospital stay (R = 0.27; $p = 0.011$ and R = 0.49; $p < 0.001$) as well as with Ranson's score on study day 2 (R = 0.39; $p < 0.001$). No correlations were observed between serum IL-6 and patients' age.

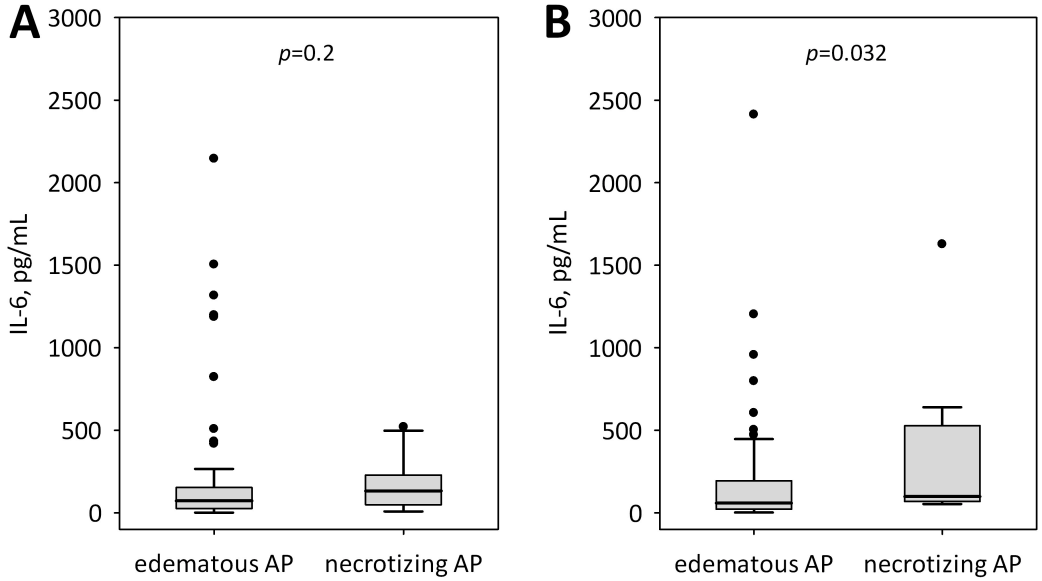

Figure 2. IL-6 concentrations at admission (**A**) and on day 2 of hospital stay (**B**) in edematous and necrotizing pancreatitis. Data are shown as median, interquartile range (box), non-outlier range (whiskers) and outliers (points).

Both on admission and on day 2 of hospital stay, serum concentrations of IL-6 correlated with other inflammatory markers (albumin, C-reactive protein, procalcitonin, white blood cells, neutrophils), markers of endothelial dysfunction (sFlt-1, Ang-2), as well as the selected laboratory markers of organ dysfunction (Table 3).

Table 3. Correlations between IL-6 and selected laboratory markers in patients with AP during first 48 h of disease.

Variables	Study day 1		Study day 2	
	R	*p*	R	*p*
sFlt-1	0.38	0.001	0.24	0.1
Ang-2	0.26	0.1	0.54	0.004
Total calcium	−0.34	0.003	−0.44	<0.001
Total protein	−0.48	0.001	−0.48	<0.001
Albumin	−0.34	0.005	−0.57	<0.001
CRP	0.35	0.001	0.67	<0.001
PCT	0.44	<0.001	0.70	<0.001
WBC	0.37	<0.001	0.50	<0.001
NEU	0.42	0.006	0.63	<0.001
Urea	0.10	0.4	0.22	0.044
KIM-1	0.50	<0.001	0.25	0.045
L-FABP	0.14	0.4	0.51	<0.001
LDH	0.27	0.020	0.63	<0.001
Bilirubin	0.09	0.4	0.30	0.005

Ang-2—angiopoietin 2; AP—acute pancreatitis; CRP—C-reactive protein; HCT—hematocrit; IL-6—interleukin 6; LDH—lactate dehydrogenase; NEU—neutrophils; PCT—procalcitonin; sFlt-1—soluble fms-like tyrosine kinase-1; WBC—white blood cells.

Among the four components of BALI score, LDH activity exceeding 300 U/L was the one most often observed (in 83% of patients), following by high IL-6 concentrations (>300 pg/mL among 17% of patients), older age (over 65 years among 14% of patients), and urea above 8.93 mmol/L (9% of patients). Overall, 3 or 4 points in BALI score was observed in 4% of the studied group.

IL-6 concentrations measured on admission and on day 2 of hospital stay significantly predicted subsequent vital organ failure with Marshall score > 2 points and the diagnosis of SAP (Table 4). Only admission IL-6 was significant predictor of ICU transfer or death (Table 4).

Table 4. Simple logistic regression models to predict severe course of AP based on serum IL-6 concentrations.

Dependent Variable	Odds Ratio (95% Confidence Interval per 100 pg/mL); *p*-Value	
	IL-6 on Admission	IL-6 on Day 2
SAP (2012 Atlanta)	1.17 (1.03-1.32); $p = 0.011$	1.18 (1.01-1.37); $p = 0.030$
Cardiovascular, lung or kidney failure	1.27 (1.09-1.48); $p = 0.002$	1.19 (1.02-1.39); $p = 0.023$
ICU transfer or death	1.22 (1.06-1.41); $p = 0.004$	1.15 (1.00-1.34); $p = 0.051$
Death	1.23 (0.06-1.43); $p = 0.007$	1.02 (0.76-1.36); $p = 0.9$

Using ROC curve analysis, we compared serum IL-6 concentrations measured on admission with BALI, Ranson's, PANC3 and BISAP scores as predictors of AP severity (Figure 3). The analysis confirmed that IL-6 on admission significantly predicted SAP diagnosis (AUC 0.753; 95% CI 0.590–0.917; $p = 0.002$), organ failure with Marshall score over 2 (AUC 0.767; 95% CI 0.578–0.956; $p = 0.006$) as well as ICU transfer or death (AUC 0.781; 95% CI 0.610–0.953; $p = 0.001$). No significant differences were observed between IL-6 and the studied multi-variable scores in prediction of SAP diagnosis according to 2012 Atlanta classification. Also, no differences between IL-6 and the studied scores were observed in prediction of cardiovascular, lung and/or renal failure (defined as Marshall score >2 points). IL-6 was significantly better than PANC3 ($p = 0.020$) and did not differ from other studied scores as a predictor of ICU transfer or death. Proposed cut-off values for IL-6 concentrations on admission are 211 pg/mL for the diagnosis of SAP (sensitivity 57%; specificity 82%); 262 pg/mL for vital organ failure (sensitivity 62%; specificity 88%); and 229 pg/mL for the prognosis of ICU transfer or death (sensitivity 57%; specificity 83%).

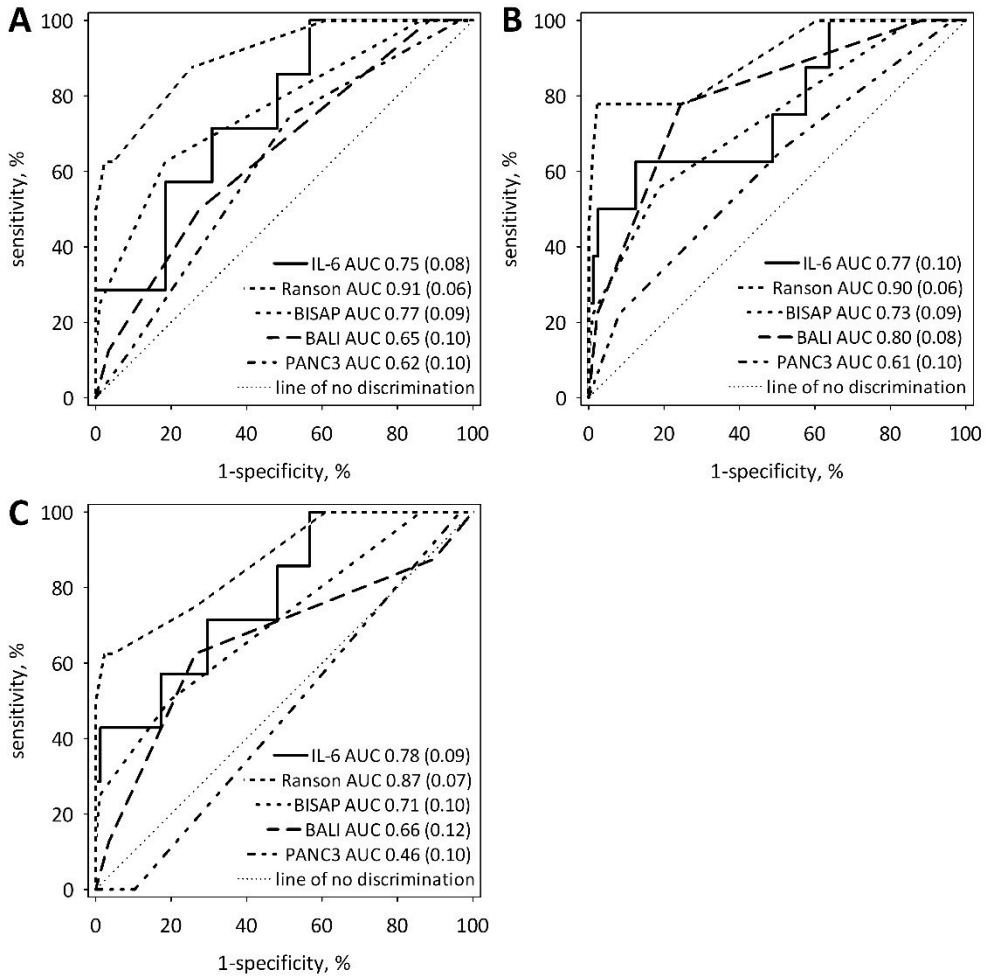

Figure 3. ROC curves showing diagnostic usefulness of IL-6 on admission (solid lines) in comparison to known predictive scores (Ranson's, BISAP, BALI, PANC3) in prediction of SAP according to 2012 Atlanta classification (**A**), organ failure with 2 or more points in Marshall score (**B**), and ICU transfer or death (**C**). The values of area under the ROC curve (AUC) with standard errors (in brackets) are shown on the graphs.

3. Discussion

The dynamic course of AP and the risk of developing systemic complications both encourage researchers to look for new early biomarkers of AP severity, re-evaluate the present ones, and verify the currently implemented prognostic scores. Important criteria for assessing the utility of predictive markers include the time needed to conduct adequate laboratory tests, but also the measurement techniques used. In the early phase of AP, life-threatening organ failure may develop during the first 48 h from the onset of symptoms. This narrows the therapeutic window and indicates the need for urgent measures to improve prognosis in predicted severe AP [30].

The majority of currently used multi-variable prognostic scales such as BISAP, Ranson's, Glasgow, PANC3, or APACHE II employ routinely available laboratory testing methods, as well as the results of clinical monitoring and imaging. Only the BALI score proposed in 2006 by Spritzer et al. [31] utilized cytokine (IL-6) measurement as a component of prognostic assessment. Very recently, it has been shown that the addition of IL-6 improves the prediction of SAP based on SIRS criteria [29]. Nonetheless, because of the limited availability of routine IL-6 tests, BALI prognostic score has been rarely implemented. Scarcely any of the laboratories have experience in defining the cut-off points for cytokine concentrations predictive of severe AP.

Initial events in AP are associated with the premature activation of pancreatic enzymes within acinar cells. There is also evidence for nuclear factor-κB activation in acinar cells resulting in the release of cytokines and chemokines [22,32,33]. The infiltration of inflammatory cells (neutrophils, lymphocytes, and monocytes) into the pancreas promotes local injury and exaggerates inflammation [34]. The peak increase in IL-6 concentrations in systemic circulation has been shown to be an early event in experimental AP, preceding the most severe injury of the pancreas [28]. In mild AP, the inflammation remains restricted to the pancreas, while moderately severe and severe cases are associated with systemic activation of immune cells, including lymphocytes, neutrophils, and monocyte/macrophage lineage, associated with an increased expression of IL-6, IL-8, macrophage migration inhibitory factor, myeloperoxidase, neutrophil elastase, or leukotriene B4 [35]. The recent study of Jain et al. [29] points towards genetic polymorphism of the IL-6 gene (−174 G/C polymorphism) as a cause of higher serum concentrations of IL-6, among patients with more severe AP. However, the decrease in lymphocyte counts is observed in SAP simultaneously with SIRS, as well as in the increase in serum levels of anti-inflammatory cytokines, including IL-10 [35]. IL-6 as an important and early responder in the inflammatory cascade, showing also anti-inflammatory properties, seems to play an important role in the disruption of the immune system observed in SAP.

Cytokines, released in pancreatic damage, belong to low-molecular-weight proteins, physiologically present in low concentration in systemic circulation. Yet, in the course of developing AP and in response to damaging factors and inflammation triggers, their dynamic and uncontrolled increase can be observed [9]. Cytokines such as IL-1β, TNF-α, or platelet-activating factor are most often directly connected with the progression of inflammation, whereas the co-occurring activity of cytokines, i.e., IL-6, IL-8, IL-10, or free radicals in the course of AP, can affect the extent of inflammation and be associated with AP prognosis [9,15,36]. The study by Kay et al. [20] suggests that the initial inflammatory response in AP develops as naive Th17 cells are generated in response to macrophage-derived IL-6 [20], whereas the development of SAP occurs as a result of Th17 response with a greater pathogenic potential (IL-23 induced), probably due to dysfunctional autophagy or the inability of monocytes to mount an adequate IL-10 anti-inflammatory response due to anergy [20]. Moreover, Kostic et al. [21] have demonstrated that IL-6, IL-8, and IL-10 levels in the first three days of AP are accurate markers of necrosis and of a potentially lethal outcome. Observations made by Kostic et al. [21] of significantly higher IL-6 levels in patients with necrotizing AP, remain consistent with the results presented in this article.

In our study, patients who subsequently developed necrotizing AP presented higher serum IL-6, however, the difference from those with edematous AP became significant on day 2 of the study. Nevertheless, IL-6 on admission significantly predicted SAP diagnosis, organ failure with a Marshall score ≥ 2, as well as ICU transfer or death. IL-6 concentrations in patients with SAP were significantly higher compared to patients with MAP and MSAP, although the difference was more significant on day 2 of hospital stay. No significant changes were observed between admission and day 2 serum IL-6 levels. IL-6 concentrations on each of the two days were positively correlated with the length of hospital stay as well as Ranson's score. Moreover, interesting correlations were observed between IL-6 and the early markers of acute kidney injury (KIM-1 and L-FABP) as well as the markers of endothelial dysfunction (Ang-2 and sFlt-1) [17,37,38].

In literature, a number of valuable studies justify the need for IL-6 assessment in the early prediction of AP severity [1,2,9,11,21,30], however, in light of contradictory information regarding the analytical aspect of IL-6 measurements and prognostic utility of different assays in the first 48 h of AP, implementation of this diagnostic procedure requires re-evaluation.

Serum IL-6 measurements are still rarely conducted on routine basis despite the availability of automatized IL-6 assay. In the present study, we evaluated the diagnostic usefulness of IL-6 test using a fully automated analytical platform operating on routine daily basis. The performance of the analytical procedure was controlled according to standard laboratory quality control practice and provided repeatable, reproducible results, in compliance with the requirements of Good Clinical and Laboratory

Practice. We believe that for the evaluation of the diagnostic usefulness of IL-6, the measurements should be fully automated, standardized, and conducted in real time. The results of IL-6 measurements obtained by means of ELISA technique are carried out in series, after the collection of entire set of specimens and although they give valuable information on the dynamics of IL-6 changes in the course of AP, they do not permit the clinician to accumulate experience based on day-to-day practice.

In order to predict the unfavorable outcome in the course of AP, we estimated the best cut-off points for IL-6. The adopted values are slightly lower in comparison to those proposed in BALI score (IL-6 > 300 pg/mL) [31]. In our study, serum IL-6 > 211 pg/mL on admission predicts SAP, whereas IL-6 > 262 pg/mL indicates the risk of vital organ failure. The optimal cut-off point for predicting ICU transfer or death is >229 pg/mL. The diagnostic accuracy of IL-6 measured on admission was not significantly different as compared to most multi-variable prognostic scores, including Ranson's score (that requires the results of multiple laboratory tests done both on admission and on the following day).

Our study is not without limitations. Firstly, the percentage of patients with MAP is small as compared to most reports, including the recent epidemiological data from Eastern Europe published by Párniczky et al. [39]. In this analysis of 600 patients with AP, 61.2% were diagnosed with MAP according to 2012 Atlanta classification, while in our study only 30.5% of patients had MAP. This is related to the practice of the hospital where our patients were recruited. In many Polish hospitals, patients with predicted mild AP are admitted to non-surgical wards (gastroenterology or internal medicine). Our study included patients admitted to surgical ward, i.e., those who presented with more severe symptoms or SIRS, developed early local complications, or had comorbidities posing a higher risk for MSAP/SAP. For this reason, our cohort is "enriched" in MSAP patients. The proportion of patients with SAP (8.5%) is comparable to the data of Párniczky et al. (8.8%) [39]. Secondly, the percentage of patients who received antibiotic treatment is high, taking into account that current guidelines (including Polish ones) do not support preventive antibiotics [4,40]. This is in part associated with the severity issue. Antibiotics were introduced empirically when patients, retarded clinically in the course of AP, developed sustained fever, or when inflammatory markers including procalcitonin significantly increased. In most patients, antibiotics were not administered during the two initial days of hospital stay, so the concentrations of studied laboratory markers were not significantly affected by this treatment. Finally, the percentage of patients diagnosed as idiopathic AP is relatively high. A part of these patients was referred for further diagnostics to the tertiary center, but we were unable to collect the follow-up data.

Still, our results indicate that automatically measured serum IL-6 is a useful biomarker in the prediction of the unfavorable course of AP in a setting of secondary care hospital, i.e., a place where most patients with AP are initially admitted. A larger multicenter study is needed to allow for a generalization of the results and wider clinical uptake of IL-6 measurements.

4. Materials and Methods

This was a prospective, observational study carried out in the setting of a secondary care hospital. The study was conducted in Surgery Unit, Complex of Health Care Centers in Wadowice, Poland from January 2014 until December 2015. Patients were recruited within 24 h of the time of hospital admission. The study included adult patients diagnosed with AP. AP diagnosis was based on the revised 2012 Atlanta classification system [19]. Patients whose symptoms lasted for longer than 24 h before admission were excluded from the study. Moreover, patients with chronic pancreatitis, disseminated neoplastic disease, pancreatic cancer, and chronic liver disease were excluded. Only the patients who gave their written informed consent to participate in the study were included. The study protocol was approved by the Bioethics Committee of the Beskidy Medical Chamber (approval number 70/2014/B issued on 6 February 2014).

The severity of AP was defined according to the revised Atlanta classification system [10,19]. Patients who did not present any local/systemic complications nor organ failure were assigned to the mild acute pancreatitis (MAP) group. Those with local/systemic complications and transient organ

failure (lasting less than 48 h) were assigned to the moderately severe acute pancreatitis (MSAP) group. The group with severe acute pancreatitis (SAP) comprised patients with persistent organ failure (which did not resolve within 48 h) in the early or late phase of the disease [19]. Vital organ (cardiovascular, respiratory, renal) failure was defined according to Marshall score [19]. Necrotizing AP was diagnosed in patients who developed (peri-) pancreatic necrosis at any time during hospitalization while those with no evidence of necrosis were diagnosed with edematous AP.

On admission, demographic data were recorded, patient's history were collected, and a detailed physical examination was done. On admission (study day 1) and on the day following admission (study day 2), all patients underwent laboratory testing, including a complete blood count, biochemical tests (i.e., albumin, total protein, total calcium, glucose, urea, creatinine, bilirubin, aminotranspherases, C-reactive protein, lactate dehydrogenase (LDH)), coagulation system and urine tests. Routine tests were conducted in a Diagnostic Laboratory in Wadowice. The data was used to compute values of selected prognostic scales (BISAP, Ranson's, PANC3, and BALI scores). The BALI score includes the following: blood urea nitrogen (BUN) $\geq$ 25 mg/dL (equal to urea $\geq$ 8.93 mmol/L), age $\geq$ 65 years; serum LDH $\geq$ 300 U/L; and serum IL-6 $\geq$ 300 pg/mL) [31]. PANC3 score comprises the following: hematocrit > 44%; pleural effusion on chest X-ray; and BMI > 30 kg/m^2 [3]. BISAP includes five components: BUN $\geq$ 25 mg/dL (or urea $\geq$ 8.93 mmol/L); impaired mental status; presence of SIRS; age > 60 years; and evidence of pleural effusion [41]. Ranson's score was computed using appropriate data from days 1 and 2 of hospital stay [10].

The serum and urine samples used in the routine laboratory testing also served for additional tests. The samples were aliquoted, frozen, and stored for a period of no longer than 6 months. The measurements of serum angiopoietin-2 (Ang-2), urine kidney injury molecule-1 (KIM-1), and urine liver-fatty acids binding protein (L-FABP) were conducted in the Department of Diagnostics, Chair of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland using ELISA reagent kits that include the following: Quantikine Angiopoietin 2 ELISA (R&D Systems, Minneapolis, MN, USA); Human Quantikine TIM-1/KIM-1/HAVCR Immunoassay from (R&D System, Minneapolis, MN, USA); and L-FABP (CMIC Holdings Co., Tokyo, Japan). For Ang-2, the minimum detectable dose was 8.29 pg/mL, and the mean serum concentration in healthy volunteers was 2494 pg/mL (range 1065–8907). For KIM-1 assay, the minimum detectable dose was 0.009 ng/mL. The normal range provided by the manufacturer was from 0.156 to 5.33 ng/mL. For L-FABP, the sensitivity of the assay was 3 ng/mL.

The concentrations of IL-6 and soluble fms-like tyrosine kinase-1 (sFlt-1) were measured by ECLIA on Cobas 8000 analyzer (Roche Diagnostics, Mannheim, Germany) in the Diagnostics Department of University Hospital in Krakow.

Statistical Analysis

Categorical data were reported as numbers (percentage). Quantitative data were reported as median (lower-upper quartile) for non-normally distributed variables and mean $\pm$ standard deviation for normally distributed variables (as assessed with Shapiro-Wilk's test). Kruskal-Wallis ANOVA or one-way ANOVA were used to study differences between groups based on AP severity. Spearman's rank correlation coefficients were used to study correlations of IL-6 (considering non-normal distribution of IL-6 concentrations). Logistic regression was used to study the association of IL-6 concentrations with outcome variables (SAP, organ failure, ICU transfer or death). ROC curves were used to assess diagnostic utility of IL-6 in comparison with multi-variable scores. The tests were two-tailed, and the results were considered significant at p-value below 0.05. The calculations were made with the use of Statistica 12 software (StatSoft, Tulsa, OK, USA).

5. Conclusions

Serum interleukin-6 (IL-6) has been proposed as a biomarker to assist in the early diagnosis of SAP, however, most data come from studies utilizing IL-6 measurements with ELISA. Our study confirms

the diagnostic usefulness of fully automatized IL-6 measurements to predict the development of SAP, vital organ failure, and the need for intensive care or death from AP. The fully automated assay allows for fast and repeatable measurements of serum IL-6, enabling the clinicians to incorporate the result in routine diagnostic reasoning.

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Artykuł nr 2

Witold Kolber, Beata Kuśnierz-Cabala, Paulina Dumnicka, Małgorzata Maraj, Małgorzata Mazur-Laskowska, Michał Pędziwiatr, Piotr Ceranowicz

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Serum Urokinase-Type Plasminogen Activator Receptor Does Not Outperform C-Reactive Protein and Procalcitonin as an Early Marker of Severity of Acute Pancreatitis

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Abstract: Severe acute pancreatitis (SAP) concerns 10–20% of acute pancreatitis (AP) patients and is associated with a poor prognosis and high mortality. An early prognosis of the unfavorable outcome, transfer to an intensive care unit (ICU) and the introduction of an adequate treatment are crucial for patients' survival. Recently, the elevated circulating urokinase-type plasminogen activator receptor (uPAR) has been reported to predict SAP with a high diagnostic accuracy among patients in a tertiary center. The aim of the study was to compare the diagnostic utility of uPAR and other inflammatory markers as the predictors of the unfavorable course of AP in patients admitted to a secondary care hospital within the first 24 h of the onset of AP. The study included 95 patients, eight with a SAP diagnosis. Serum uPAR was measured on admission and in the two subsequent days. On admission, uPAR significantly predicted organ failure, acute cardiovascular failure, acute kidney injury, the need for intensive care, and death. The diagnostic accuracy of the admission uPAR for the prediction of SAP, organ failure, and ICU transfer or death was low to moderate and did not differ significantly from the diagnostic accuracy of interleukin-6, C-reactive protein, procalcitonin, D-dimer and soluble fms-like tyrosine kinase-1. In the secondary care hospital, where most patients with AP are initially admitted, uPAR measurements did not prove better than the currently used markers.

Keywords: urokinase-type plasminogen activator receptor; interleukin 6; early prediction of acute pancreatitis severity; acute kidney injury; liver failure

1. Introduction

Acute pancreatitis (AP) is one of the most common acute digestive tract diseases and, despite significant medical advancement in the last decade, it still poses a risk of life-threatening

complications. In case of severe acute pancreatitis (SAP), mortality can reach 20–30% [1–3]. Recent studies indicate that in consequence of the systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) nearly half of deaths occur within the first week of AP [1,4,5]. These findings have been reflected in the 2012 revised Atlanta classification [6], which defines the early and the late phases of the disease. The early phase covers the period of one week from the onset of symptoms and is followed by the late phase lasting weeks or even months. The first 48 h are important for the further course of AP as adequate clinical management (including intensive fluid resuscitation) during the so-called therapeutic window can reduce the risk of complications [1,3,4,7]. This is especially important for patients developing MODS who should be referred to an intensive care unit (ICU) [7]. The diagnostic process to assess AP severity should take place in the first 48 h from the onset of symptoms. At present, it involves clinical assessment, imaging tests, multi-variable predictive scores (Ranson, APACHE II, Glasgow, BISAP) and laboratory testing [1,3,7,8]. Unfortunately, none of the above diagnostic tools appears flawless.

The laboratory tests that have been proposed for the assessment of AP severity include proinflammatory [tumor necrosis factor-α (TNF-α), interleukins (IL): IL-6, IL-1β, IL-18, IL-8] and anti-inflammatory (IL-4, IL-10, IL-1 receptor antagonist) mediators, soluble receptors (soluble TNF-α receptor II), microRNAs, adhesive molecules (soluble intercellular adhesion molecule 1), growth factors (hepatocyte growth factor, tumor growth factor-β1), procalcitonin, and acute phase proteins [C-reactive protein (CRP), serum amyloid A] [5,9–11]. However, many of the above markers can only be measured with enzyme-linked immunoassays rarely conducted in real time because of long procedures and high costs when not applied in series. At present, no single laboratory marker can be recommended for AP severity stratification, although in the clinical practice CRP measurements are often used [4]. Moreover, IL-6 and procalcitonin can be useful, as patients with SAP present with clearly higher concentrations in the first 48 h from the onset of symptoms: $\geq$300 pg/mL [12] and $\geq$3.3 ng/mL, respectively [9,10,13].

Patients with SIRS of either infectious or non-infectious origin present a severe endothelial dysfunction and hypercoagulability [14–16]. Endothelial dysfunction, together with tissue hypoperfusion, lead to gradual progression of organ failure [5,14,17]. The process is accompanied by the activation of coagulation and fibrinolysis, reflected in altered results of laboratory coagulation tests, e.g., high D-dimer concentrations [16,18].

One of the earliest fibrinolysis mediators is the urokinase-type plasminogen activator (uPA) [17]. Its ability to convert plasminogen into plasmin is potentiated by binding to the uPA receptor (uPAR, CD87) expressed on the endothelial cells, monocytes, macrophages, T-cells and granulocytes [17,19–21]. Besides being involved in fibrinolysis, uPAR assists in cell adhesion, migration, chemotaxis, immune activation, and tissue reconstruction [22,23]. The protein is bound to cell membranes by glycosylphosphatidylinositol anchor and may be shed or released into body fluids [23]. Soluble uPAR is present in plasma or serum, urine and cerebrospinal fluid [20–23]. Increased serum uPAR has been associated with various inflammatory states, particularly with sepsis and non-septic SIRS [17,20–22]. The correlation with severity of organ failure and mortality, together with the relative stability of plasma or serum concentrations, make uPAR a promising clinical biomarker in patients with SIRS [20,22,24,25], but so far has not been extensively studied in AP. Nonetheless, in 2017, Lipinski et al. [26] showed a very high diagnostic accuracy of plasma uPAR for the prediction of SAP and fatal AP among patients of a tertiary referral hospital.

The aim of our study was to assess the diagnostic usefulness of uPAR in the prediction of severe course of AP in a secondary care hospital setting, where most patients with AP are initially admitted. We were interested to know whether uPAR testing may help in the early (preferably within the first 48 h from the onset of AP symptoms) prediction of organ failure and, thus, the need for ICU transfer, and whether in this regard uPAR outperforms other single laboratory markers associated with AP severity.

2. Materials and Methods

The prospective observational study was designed to test whether the concentrations of uPAR measured in serum on the first days of AP allow the prediction of disease severity assessed during the hospital stay, and to compare uPAR with the other single laboratory tests used to predict AP severity. The study included adult patients admitted with AP, treated at the Surgery Ward, Complex of Health Care Centers in Wadowice, Poland (a secondary care hospital). Consecutive patients with a diagnosis of AP who were admitted to the Ward between March 2014 and December 2015 were assessed regarding eligibility for the study. Only patients with symptoms of AP lasting up to 24 h before hospital admission were included in the study. The exclusion criteria were chronic pancreatitis, known malignancy, and chronic liver disease. The patients provided written informed consent for the study. The study included patients who were able to provide an informed consent within a day of admission. The patients included in the study were asked to give three blood samples for laboratory tests, one sample on each of the first three days of the hospital stay. The demographic and clinical data were collected, including the etiology of AP, data on complications, severity and treatment of AP during the hospital stay. The main outcome variables were the organ failure during the first week of the hospital stay, the severity of AP defined according to the 2012 Atlanta classification (based on the entire course of the hospital stay) [6], and the ICU transfer or death during the hospital stay. The study protocol was approved by the Bioethics Committee of the Beskidy Medical Chamber (approval number 2014/02/06/1 issued on 6 February 2014).

The diagnosis of AP was based on the 2012 revised Atlanta classification [6], when at least two out of three of the following criteria were positive, i.e., typical clinical presentation of AP, serum amylase or lipase activity three times the upper reference value, and typical findings in dynamic computed tomography or magnetic resonance imaging or ultrasonography. The severity of AP was classified according to the 2012 Atlanta classification [6] as mild acute pancreatitis (MAP), moderately severe acute pancreatitis (MSAP) or severe acute pancreatitis (SAP). Organ failure was identified using the Modified Marshall Scoring System (MMSS) [6]. The diagnosis of acute kidney injury (AKI) was based on the Kidney Disease Improving Global Outcomes (KDIGO) criteria [27]. The diagnosis of acute respiratory distress syndrome (ARDS) was based on the Berlin criteria [28].

On admission (day 1), and on the two following days (day 2 and 3), blood samples were obtained from patients for routine blood tests and for measurements of uPAR, IL-6 and soluble fms-like tyrosine kinase-1 (sFlt-1). The routine blood tests included complete blood cell count, serum concentrations of urea, creatinine, bilirubin, CRP and procalcitonin, serum activities of amylase, lactate dehydrogenase (LDH), aspartate and alanine aminotransferases (AST, ALT), and plasma concentrations of D-dimer. The routine tests were conducted on the day of blood collection in the Central Laboratory, Complex of Health Care Centers in Wadowice, Poland using automatic analyzers: Sysmex XN (Sysmex Corporation, Cobe, Japan) for the blood counts, Cobas E411 (Roche Diagnostics, Mannheim, Germany) and Vitros 5600 (Ortho Clinical Diagnostics, Raritan, NJ, USA) for biochemistry and immunochemistry, and Coag XL (Diagon, Budapest, Hungary) for the coagulation testing. The performance of routine laboratory tests was assessed with daily internal quality control and regular external quality control (including Centre for Quality Assessment in Laboratory Medicine in Poland and Randox International Quality Assessment Scheme, RIQAS), in line with good laboratory practice. Additional serum samples for IL-6, uPAR and sFlt-1 assessment were aliquoted and frozen in -80°C. IL-6 and sFlt-1 were measured by electrochemiluminescence immunoassay (ECLIA) on Cobas 8000 analyzer (Roche Diagnostics, Mannheim, Germany) in the Diagnostic Department of University Hospital in Krakow, Poland. The minimum detectable doses were 1.5 pg/mL for IL-6 and 10 pg/mL for sFlt-1. The tests were calibrated according to the manufacturer's instruction, the measurements in patients' samples were run in series and were preceded by controls (PreciControl Multimaker, Roche, Mannheim, Germany) on two levels. For the IL-6 test, the intraassay precision was $\leq$6.0% and the interassay precision was $\leq$8.5%, and for the sFlt-1 test, the intraassay precision was $\leq$3.9 and the interassay precision was $\leq$5.6%, as reported by the manufacturer of the tests. Serum uPAR

was measured with enzyme-linked immunoassay, using Quantikine Human uPAR Immunoassay reagent kit (R&D Systems, Minneapolis, MN, USA) in the Department of Diagnostics, Chair of Clinical Biochemistry at Jagiellonian University, Krakow, Poland. The minimum detectable dose of uPAR was 0.033 ng/mL, the reference range provided by the manufacturer was 1.195–4.415 ng/mL. Patients' samples were tested for uPAR in duplicates, and in series; each run was calibrated according to the manufacturer's instructions. The intraassay precision for the test was $\leq$7.5% and the interassay precision $\leq$5.6%.

Nominal data were reported as number (percentage of the group). Quantitative data were reported as mean and standard deviation (SD) or median, lower and upper quartiles (Q1; Q3), depending on normality of each variable's distribution (as assessed with Shapiro-Wilk's test). The contingency tables were analyzed with Pearson's chi-squared test. In case of three groups, the whole contingency table was analyzed first, and then the pairwise comparisons were done using Pearson chi-squared test with Bonferroni correction. Due to the non-normal distribution of most quantitative variables, Kruskal-Wallis's analysis of variance (with post-hoc comparisons using Siegel and Castellan method) was applied when three groups were compared and Mann-Whitney's test when two groups were compared. Spearman's rank order coefficient was used to assess correlations. Simple and multiple logistic regression was used to assess uPAR as a predictor of unfavorable course of AP. Receiver operating characteristic (ROC) curves were used to evaluate the diagnostic accuracy of the studied laboratory tests; the cut-off values were selected by maximizing the Youden index. The statistical tests were two-tailed, and the results were considered significant at $p < 0.05$. Statistica 12 software (Statsoft, Tulsa, OK, USA) was used for computations.

3. Results

The study included 95 patients with AP, 30 women, 65 men, with a mean (SD) age of 48 (16) years. According to the 2012 Atlanta criteria, 29 patients (31%) were diagnosed with MAP, 58 (61%) with MSAP and 8 (8%) with SAP. MAP, MSAP and SAP patients did not differ significantly in terms of age, sex, percentage affected with comorbidities, and AP etiology (Table 1). Average Ranson's score was higher among patients with MSAP and SAP, while SIRS was common among both MSAP and SAP patients. The patients' treatment (the need for surgery, nutritional support and ICU transfer) reflected the severity of AP. Four patients (4%) died—one in the early and three in the late phase of AP (Table 1).

On admission, the patients with SAP were characterized with significantly higher concentrations of urea, creatinine, glucose and IL-6 (Table 2). Other studied laboratory markers did not differ significantly between the MAP, MSAP and SAP patients on admission, although the median concentrations of inflammatory markers: Serum uPAR, CRP, procalcitonin and plasma D-dimer, as well as the median numbers of leukocytes were relatively high among patients with SAP. This was also observed in the case of endothelial dysfunction marker, i.e., sFlt-1 (Table 2).

Serum uPAR concentrations were significantly higher in women as compared to men, starting from the day of admission (Figure 1). No sex-related differences were observed for any other inflammatory markers (IL-6: $p > 0.5$; procalcitonin: $p > 0.2$; D-dimer: $p > 0.1$; white blood cells: $p > 0.1$; admission and day 3 CRP: $p > 0.1$), except for the day 2, when CRP was higher in men (median 315 in men versus 185 mg/L in women; $p = 0.011$). Also, no significant differences were observed between men and women regarding the severity of AP as defined according to the Atlanta classification (Table 1). Serum uPAR concentrations did not differ between patients with AP of various etiology ($p = 0.7$ on admission and day 2; $p = 0.2$ on day 3). No correlations were observed between uPAR and age. Patients with comorbid conditions (mainly ischemic heart disease) tended to present higher concentrations of uPAR, however, the difference was only significant on day 2 of the study: Median (Q1; Q3) was 3.77 (2.97; 5.03) ng/mL in those affected by comorbidities versus 2.93 (2.54; 3.88) ng/mL in the patients without comorbidities ($p = 0.023$).

Table 1. Clinical characteristics of the study group according to the severity of acute pancreatitis (AP).

Characteristic	MAP (*n* = 29)	MSAP (*n* = 58)	SAP (*n* = 8)	*p*-Value
Male sex, *n* (%)	17 (59)	41 (71)	7 (88)	0.2
Mean age (SD), years	43 (16)	50 (16)	51 (20)	0.1
Pre-existing comorbidities, *n* (%)	10 (34)	27 (47)	5 (62)	0.3
Cardiac diseases, *n* (%)	5 (17)	20 (34)	5 (62)	
Diabetes, *n* (%)	0	6 (10)	2 (25)	
Dyslipidemia, *n* (%)	1 (3)	2 (3)	0	
Chronic kidney disease, n (%)	0	2 (3)	0	
Liver disease, *n* (%)	1 (3)	2 (3)	0	
Other comorbidities, *n* (%)	3 (10)	0	0	
Etiology				0.1
Biliary, *n* (%)	9 (31)	17 (29)	1 (12)	
Alcoholic, *n* (%)	12 (41)	11 (19)	6 (75)	
Hipertriglyceridemia, *n* (%)	1 (3)	4 (7)	0	
Other/idiopathic, *n* (%)	7 (24)	26 (45)	1 (12)	
Median Ranson score (Q1; Q3), points	2 (1; 3)	3 (3; 4)	6 (4; 7)	<0.001 [a,b,c]
Median duration of hospital stay(Q1; Q3), days	10 (7; 12)	14 (10; 16)	26 (13; 41)	0.001 [a,c]
SIRS in first 24 h, *n* (%)	18 (62)	49 (84)	7 (88)	0.047 [c]
Early/late mortality, *n* (%)	0	0/2 (3)	1 (12)/1 (12)	0.006 [a,b]
Therapeutic ERCP, *n* (%)	0	3 (5)	2 (25)	0.020 [a,b]
Surgery, *n* (%)	0	3 (5)	4 (50)	<0.001 [a,b]
Enteral feeding via nasojejunal tube, *n* (%)	0	4 (7)	6 (75)	<0.001 [a,b]
Parenteral feeding, *n* (%)	0	1 (2)	2 (25)	0.001 [a,b]
Transfer to ICU, *n* (%)	0	2 (3)	5 (62)	<0.001 [a,b]

ERCP, endoscopic retrograde cholangiopancreatography; ICU, intensive care unit; MAP, mild acute pancreatitis; MSAP, moderately severe acute pancreatitis; n, number of patients; SAP, severe acute pancreatitis; SD, standard deviation; SIRS, systemic inflammatory response syndrome; Q1, lower quartile; Q3, upper quartile; *p*-value is reported for overall comparison between three groups (in Pearson chi-squared test or Kruskal-Wallis ANOVA), the letters in superscript indicate the results of post-hoc tests: [a] significant difference between the MAP and SAP groups in post-hoc comparison; [b] significant difference between the MSAP and SAP groups in post-hoc comparison; [c] significant difference between the MAP and MSAP groups in post-hoc comparison.

Table 2. The results of laboratory tests on admission according to the AP severity. Data are shown as mean (SD) or median (Q1; Q3).

Variable	MAP (*n* = 29)	MSAP (*n* = 58)	SAP (*n* = 8)	*p*-Value
Hematocrit, %	42 (5)	43 (6)	46 (7)	0.4
Albumin, g/L	38 (7)	35 (6)	36 (8)	0.6
Total calcium, mmol/L	2.13 (0.23)	2.15 (0.19)	1.92 (0.48)	0.5
Glucose, mmol/L	6.44 (5.61; 7.67)	8.17 (6.78; 9.33)	7.92 (7.22; 10.64)	0.002 [a,c]
Urea, mmol/L	3.67 (2.83; 6.00)	4.67 (3.50; 6.00)	6.67 (5.00; 13.00)	0.015 [a]
Creatinine, µmol/L	65.4 (59.2; 80.4)	69.8 (60.1; 87.5)	92.4 (75.6; 171.1)	0.033 [a]
Bilirubin, µmol/L	23.4 (13.5; 38.5)	27.2 (13.8; 53.3)	29.1 (16.2; 36.9)	0.7
AST, U/L	59 (33; 209)	116 (55; 202)	122 (87; 166)	0.3
ALT, U/L	62 (43; 174)	133 (54; 299)	85 (49; 158)	0.3
LDH, U/L	553 (488; 810)	636 (507; 850)	1013 (737; 1294)	0.1
WBC, $\times 10^3/\mu L$	12.4 (9.5; 15.2)	13.1 (10.4; 16.2)	17.1 (10.3; 23.3)	0.4
Platelet count, $\times 10^3/\mu L$	199 (176; 231)	218 (165; 279)	227 (162; 292)	0.8
CRP, mg/L	22.7 (5.3; 132.4)	25.4 (11.9; 174.7)	129.6 (17.4; 316.7)	0.4
D-dimer, mg/L	1.49 (0.85; 2.19)	1.90 (1.00; 3.41)	2.76 (1.20; 3.39)	0.2
Procalcitonin, ng/mL	0.10 (0.05; 0.55)	0.17 (0.10; 0.36)	0.61 (0.14; 1.03)	0.1
uPAR, ng/mL	3.92 (2.86; 5.02)	3.81 (2.98; 5.10)	5.00 (3.41; 6.74)	0.4
Interleukin 6, pg/mL	64.7 (14.8; 95.7)	78.9 (27.8; 163.0)	210.7 (73.1; 21.5)	0.037 [a]
sFlt-1, pg/mL	129 (119; 169)	140 (112; 154)	191 (155; 536)	0.1

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; MAP, mild acute pancreatitis, MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; sFlt-1, soluble fms-like tyrosine kinase-1; uPAR, urokinase-type plasminogen activator receptor; WBC, white blood cells; *p*-value is reported for overall comparison between three groups (in Kruskal-Wallis ANOVA), the letters in superscript indicate the results of post-hoc tests: [a] significant difference between the MAP and SAP groups in post-hoc comparison; [b] significant difference between the MSAP and SAP groups in post-hoc comparison; [c] significant difference between the MAP and MSAP groups in post-hoc comparison.

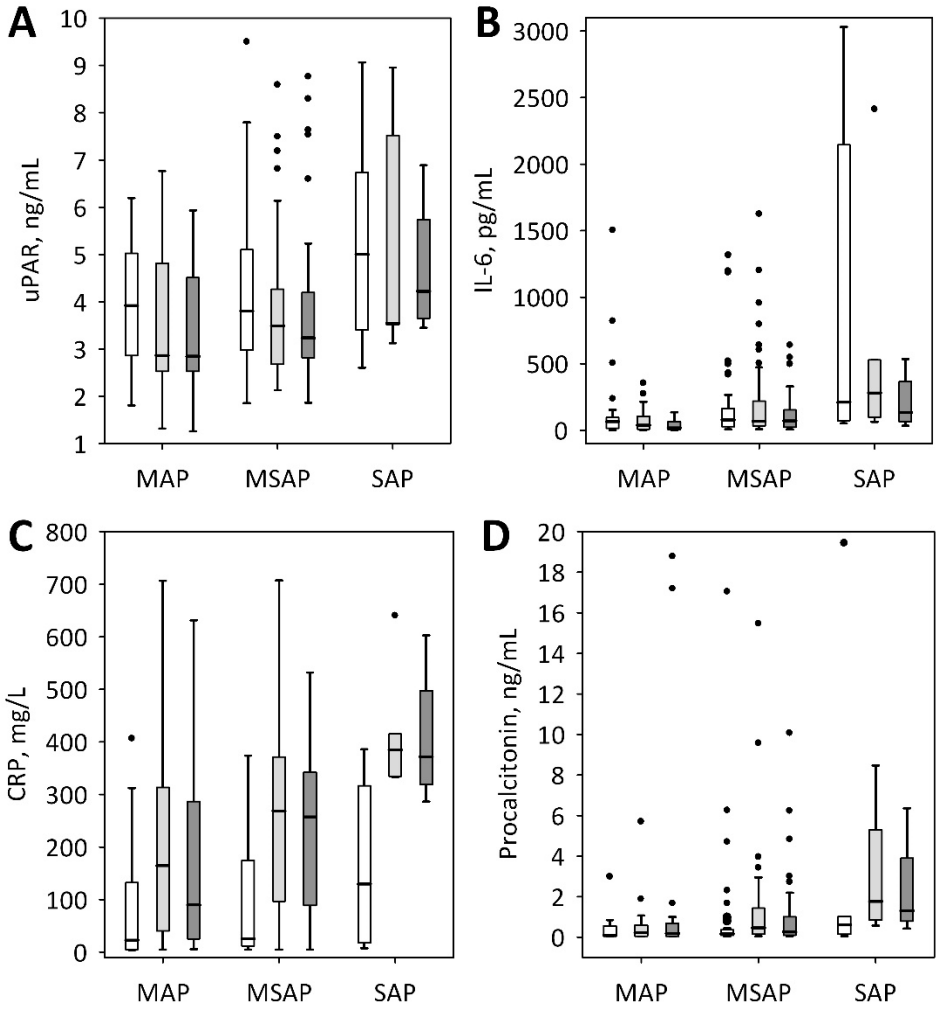

Figure 1. Serum concentrations of urokinase-type plasminogen activator receptor (uPAR) (**A**) among patients with mild (MAP), moderately-severe (MSAP) and severe acute pancreatitis (SAP) on admission (i.e., day 1 of the study, white bars), on day 2 (light grey bars) and day 3 (dark grey bars) of the hospital stay. Serum concentrations of interleukin-6 (IL-6) (**B**), C-reactive protein (CRP) (**C**), and procalcitonin (**D**) are shown for comparison. Data are presented as median, interquartile range (bars), non-outlier range (whiskers), and outliers (points).

On days 2 and 3, serum uPAR concentrations were lower as compared to the admission levels, irrespective of AP severity (Figure 2A; $p < 0.001$ in MAP and MSAP groups; $p = 0.039$ in SAP). This contrasted with the other inflammatory markers, i.e., IL-6 (no significant changes), CRP (significant increase on day 2 and 3 comparing to admission concentrations), and procalcitonin (increase in MSAP and SAP patients) (Figure 1B–D). Although the patients with SAP tended to present higher serum concentrations of uPAR (Figure 1A), the differences between SAP and MAP or MSAP patients were non-significant throughout the study period. Also, no significant differences were observed between patients with edematous and necrotizing pancreatitis ($p = 0.8$ on day 1, $p = 0.6$ on day 2 and $p = 0.8$ on day 3 of the study). However, serum uPAR concentrations measured on admission significantly predicted organ failure (defined as two or more points in the modified Marshall scoring system, i.e., in line with the 2012 Atlanta classification), acute cardiovascular failure, acute kidney injury, the need for intensive care, and death (Table 3). Moreover, the maximum of the three measurements of uPAR (from admission until day 3 of the hospital stay) significantly predicted the same complications (Table 3). In the multiple logistic regression, the associations between both, the admission and the maximum uPAR and organ failure, acute cardiovascular failure, acute kidney injury, ICU transfer and

death were all significant after adjustment for sex and the presence of comorbidities. Admission uPAR was a weak predictor of Ranson's score of ≥3 points (Table 3) and it positively correlated with the Ranson's score (R = 0.27; $p = 0.012$).

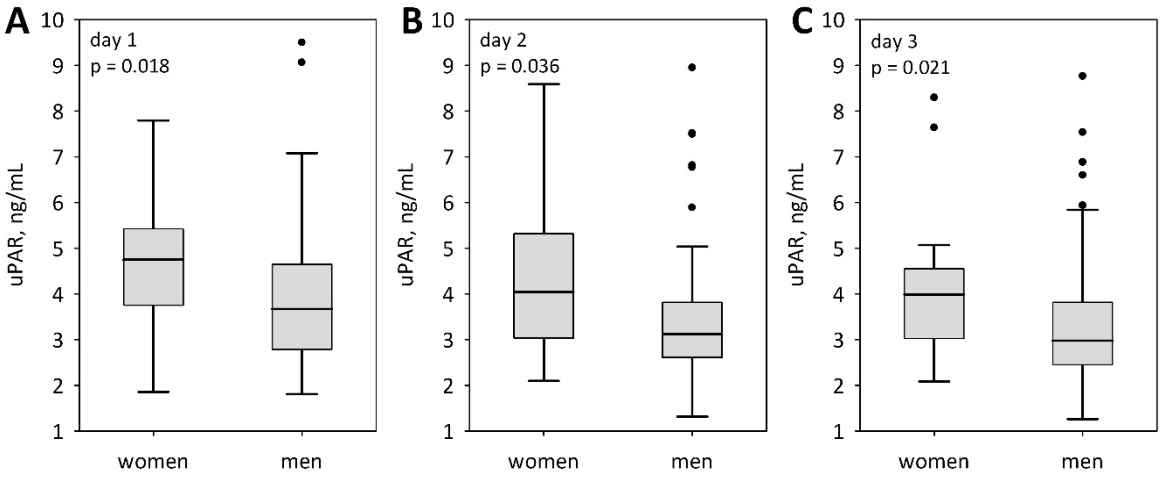

Figure 2. Sex-related differences in serum concentrations of uPAR among patients with AP during the first three days of hospital stay: On admission (day 1) (**A**), on day 2 (**B**), and on day 3 (**C**). Data are presented as median, interquartile range (bars), non-outlier range (whiskers), and outliers (points).

Table 3. Odds ratios (95% confidence intervals) for serum uPAR in prediction of unfavorable course of AP.

Dependent Variable	uPAR on Admission, per 1 ng/mL	Maximum uPAR, per 1 ng/mL
SAP (2012 Atlanta)	1.41 (0.92–2.17); $p = 0.1$	1.49 (0.94–2.37); $p = 0.08$
MSAP plus SAP (2012 Atlanta)	1.16 (0.84–1.60); $p = 0.4$	1.16 (0.86–1.58); $p = 0.3$
Persistent (≥48 h) SIRS	0.92 (0.69–1.22); $p = 0.5$	0.90 (0.69–1.18); $p = 0.4$
Ranson ≥ 3 points at 48 h	1.39 (1.01–1.89); $p = 0.038$	1.28 (0.96–1.71); $p = 0.08$
Organ failure (MMSS ≥ 2 points)	2.14 (1.33–3.46); $p = 0.002$	2.06 (1.30–3.26); $p = 0.002$
Cardiovascular failure	2.33 (1.34–4.08); $p = 0.002$	2.41 (1.29–4.50); $p = 0.005$
ARDS	1.01 (0.59–1.72); $p = 0.9$	1.19 (0.73–1.94); $p = 0.5$
AKI	1.78 (1.11–2.84); $p = 0.015$	1.77 (1.10–2.85); $p = 0.017$
ICU transfer	2.06 (1.24–3.43); $p = 0.005$	2.35 (1.27–4.35); $p = 0.005$
Death	1.82 (1.06–3.12); $p = 0.027$	2.25 (1.15–4.37); $p = 0.015$

AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; ICU, intensive care unit; MMSS, modified Marshall scoring system; MSAP, moderately severe acute pancreatitis; SAP, severe acute pancreatitis; SIRS, systemic inflammatory response syndrome; uPAR, urokinase-type plasminogen activator receptor.

We compared the diagnostic accuracy of serum uPAR for the prediction of the unfavorable course of AP with the accuracy of other proposed single markers. On admission, no significant differences were observed in the areas under the ROC curves between uPAR and IL-6, CRP, procalcitonin, D-dimer and sFlt-1 in prediction of SAP, vital organ failure and ICU transfer or death (Figure 3). Again, in this analysis, uPAR was not a significant predictor of SAP (Table 4). However, uPAR significantly predicted organ failure and ICU transfer or death. The selected cut-off values (chosen by maximizing Youden index) allowed for high specificity at relatively low sensitivity (Table 4). For comparison, the areas under the ROC curves for IL-6, CRP, procalcitonin, D-dimer and sFlt-1 are shown in Appendix A (Table A1). The diagnostic accuracy of uPAR measured on day 2 and 3, or the maximum uPAR, were not significantly better as compared to the measurements on admission, although the area under the ROC curve for uPAR measured on day 3 of the study (72 h from the onset of symptoms) was significantly different from 0.5: 0.754 (0.595–0.913) ($p = 0.002$).

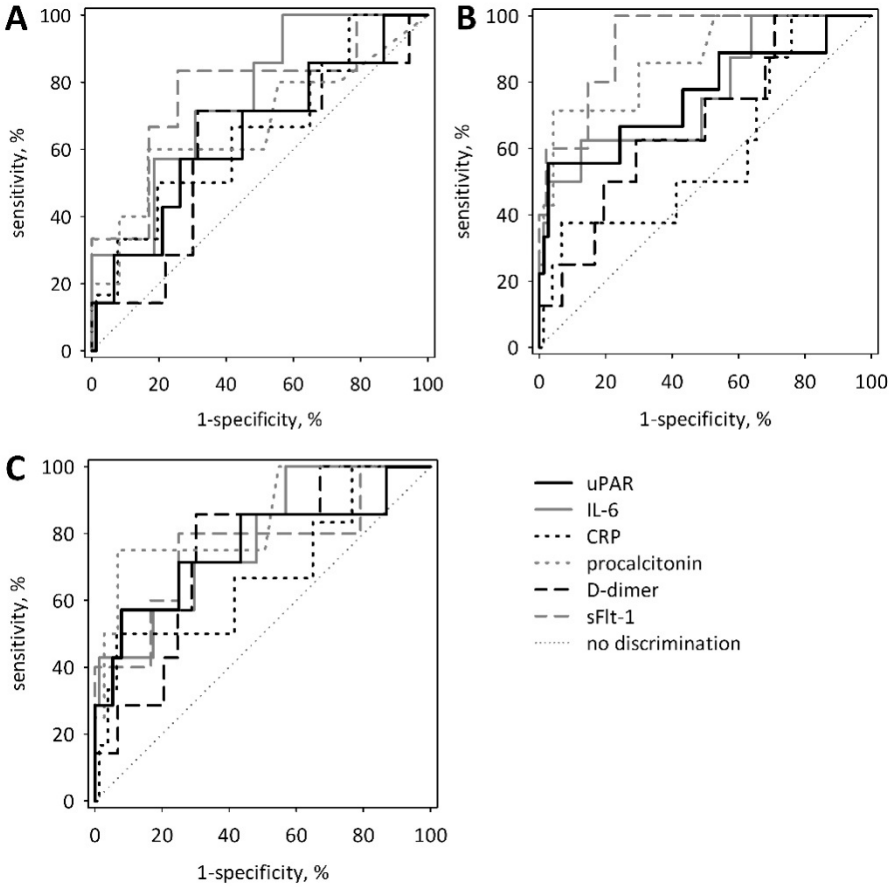

Figure 3. Receiver operating characteristic (ROC) curves for serum concentrations of uPAR on admission in prediction of SAP (**A**), vital organ failure (MMSS ≥2 points) (**B**), and ICU transfer or death (**C**). For comparison, ROC curves are shown for other proposed single biomarkers of AP severity measured on admission: Interleukin-6 (IL-6), C-reactive protein (CRP), procalcitonin, D-dimer, and soluble fms-like tyrosine kinase-1 (sFlt-1).

Table 4. Diagnostic accuracy of serum uPAR concentrations measured on admission for prediction of unfavorable course of AP.

Dependent Variable	AUC (95% CI)	*p* *	Cut-Off Value, ng/mL	Sensitivity, %	Specificity, %
SAP	0.641 (0.417–0.864)	0.2	5.004	57	75
Organ failure (MMSS ≥2 points)	0.761 (0.565–0.958)	0.009	6.736	56	97
ICU transfer or death	0.759 (0.536–0.983)	0.023	6.021	57	92

AUC, area under the receiver operating characteristic curve; CI, confidence interval; ICU, intensive care unit; MMSS, modified Marshall scoring system. * *p*-value in comparison with AUC = 0.5.

During the studied period, uPAR concentrations correlated with the other inflammatory markers, i.e., CRP (starting from day 2 of the hospital stay), procalcitonin, and IL-6 (Table 4). Positive correlation was observed between uPAR and sFlt-1 on admission and between uPAR and D-dimer on days 1 and 3. Serum uPAR negatively correlated with albumin (throughout the study) and hematocrit (starting from day 2). Moreover, uPAR correlated positively with bilirubin, aminotransferases and lactate dehydrogenase. No significant correlations were observed between uPAR and serum urea or creatinine concentrations (Table 5).

Table 5. Correlations of uPAR concentrations with selected laboratory markers during early stage of acute pancreatitis.

Variable	Day 1		Day 2		Day 3	
	R	*p*	R	*p*	R	*p*
Hematocrit	−0.11	0.3	−0.31	0.006	−0.27	0.022
Albumin	−0.32	0.014	−0.54	<0.001	−0.44	<0.001
Urea	0.16	0.2	0.10	0.4	−0.04	0.8
Creatinine	0.18	0.1	0.05	0.7	−0.05	0.7
Bilirubin	0.34	0.003	0.39	<0.001	0.41	<0.001
AST	0.49	<0.001	0.53	<0.001	0.39	0.001
ALT	0.37	0.001	0.37	0.001	0.30	0.009
LDH	0.46	<0.001	0.28	0.026	0.52	<0.001
WBC	−0.03	0.8	−0.03	0.8	0.19	0.09
CRP	0.19	0.1	0.29	0.012	0.42	<0.001
D-dimer	0.26	0.030	0.19	0.09	0.30	0.010
Procalcitonin	0.57	<0.001	0.54	<0.001	0.61	<0.001
Interleukin 6	0.25	0.027	0.35	0.002	0.54	<0.001
sFlt-1	0.48	<0.001	0.02	0.9	-	-

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; sFlt-1, soluble fms-like tyrosine kinase-1; uPAR, urokinase-type plasminogen activator receptor; WBC, white blood cells.

4. Discussion

The early identification of patients who are at risk of AP complicated by organ failure enables the provision of better care and allows for the proper allocation of intensive care resources. Despite intensive studies, we still lack laboratory markers which would allow for the early and accurate prediction of organ failure in AP. The widely adopted guidelines of the International Association of Pancreatology and the American Pancreatic Association recommend SIRS and the persistent (lasting $\geq$48 h) SIRS as early markers of severe AP, however, they also acknowledge other multiparameter scores and single laboratory markers (including CRP and procalcitonin). The guidelines emphasize the need for a repeated clinical assessment which takes into account risk factors (advanced age, comorbidities, obesity), clinical signs, and response to treatment [7]. At present, no single laboratory marker can be recommended for the early prediction of AP severity.

In the current study, we evaluated the soluble uPAR, a promising biomarker previously associated with mortality and severity of acute inflammatory conditions. Although serum uPAR measured within the first 24 h from the onset of AP symptoms significantly predicted the complicated course of AP, including the need for ICU transfer and death, its diagnostic accuracy did not appear better than that of other inflammatory markers (IL-6, procalcitonin, CRP) nor the markers associated with endothelial dysfunction and hypercoagulability (sFlt-1, D-dimer).

The soluble uPAR is considered to be a non-specific marker of inflammation, including SIRS, both the one that is related and unrelated to infection. Yu et al. [24] and Koch et al. [20] reported positive associations between circulating uPAR and proinflammatory cytokines (IL-6, TNF-α), procalcitonin and CRP. Such correlations were also observed in our study. Among patients treated in an ICU, high uPAR concentrations in serum or plasma have been associated with the severity of organ failure evaluated using the Sequential Organ Failure Assessment (SOFA), the Simplified Acute Physiology Score (SAPS) or the Acute Physiology and Chronic Health Evaluation (APACHE II) scores [17,20,21]. Circulating uPAR has been shown to predict mortality in patients with SIRS [17,29,30], cardiovascular diseases [31–33], ARDS [34] and sepsis [22,35]. Our results also show significant associations between the serum uPAR and the subsequent organ failure, the need for ICU treatment and death. In our patients, uPAR significantly predicted AKI and cardiovascular failure, but not ARDS.

We were able to identify only two previous reports regarding circulating uPAR concentrations in AP. Nikkola et al. [36] evaluated soluble uPAR as a predictor of alcoholic AP severity.

Excessive alcohol consumption is the cause of nearly 40% of AP cases [2], and uPAR has been previously shown to be elevated in alcoholic liver disease [37]. Nikkola et al. [36] reported significantly higher plasma uPAR among patients classified as non-mild AP (MSAP and SAP) versus those with MAP, however, they used plasma samples collected from day 1 until day 4 from admission. They estimated that uPAR at a cut-off value of 5.0 ng/mL can predict non-mild AP with the sensitivity of 79% and the specificity of 78%; the area under the ROC curve was 0.81 (0.70–0.92) [36]. This diagnostic accuracy is higher than the observed in our study, however, our data are based on results obtained on admission, within the first 24 h from the onset of AP symptoms. Of note, in our study uPAR did not differ between patients with AP of various etiology, in particular, no differences were observed between those with alcoholic and biliary etiology. Lipinski et al. [26] studied plasma uPAR in patients with AP of various etiologies admitted to the tertiary referral hospital. They reported a very high diagnostic accuracy of plasma uPAR for the prediction of SAP: The area under the ROC curve was 0.993 (0.983–1.000), the diagnostic sensitivity 97% and the specificity 93% at the cut-off value of 4.75 ng/mL. In addition, they reported high diagnostic accuracy of uPAR for the prediction of multiple organ failure and death: Areas under the ROC curves of 0.951 (0.951–0.991) and 0.917 (0.882–1.000), respectively [26]. Moreover, uPAR >3.65 ng/mL allowed for the discrimination between MAP and MSAP with the 80% sensitivity and 92% specificity; the area under the ROC curve was 0.928 (0.883–0.972) [26]. The results are thus more optimistic than obtained by Nikkola et al. [36]. Obviously, our present study failed to replicate the results of Lipinski et al. [26]. There may be several reasons for this. Their study population differed from ours in the percentages of patients with SAP (26% versus 8%) and MSAP (29% versus 61%). The high percentage of patients with SAP in the group of Lipinski et al. [26] reflects the population of tertiary hospital patients. We can speculate that the severity of organ failure in that population may have also been higher than in our group, as the most severe cases are quickly transferred to tertiary centers. Consequently, the absolute number of patients with SAP reported by Lipinski et al. was substantially higher than in our group (33 versus 8 patients). The low number of patients with SAP in our group may in fact be responsible for the lack of a significant difference in uPAR concentrations between SAP and less severe AP, however, we also did not observe a significant difference between the MAP and MSAP patients, that was highly significant in the study of Lipinski et al. [26]. In our group, the percentage of patients with SAP is comparable to the recent reliable epidemiological data from Eastern Europe [2], but the high percentage of patients with MSAP in our group requires explanation. This is related to the arrangement of care in AP, often used in Polish secondary hospitals. We recruited patients of the surgical ward, while those with the predicted mild AP were admitted to the internal medicine/gastroenterology wards. In addition, the studies of Nikkola et al. [36], Lipinski et al. [26] and ours utilized different reagents and different samples for the measurements of uPAR: Nikkola et al. used plasma samples, but did not specify the anticoagulant. Lipinski et al. used EDTA-anticoagulated plasma, and we used serum samples. We measured uPAR with an enzyme-linked immunoassay, and although we followed good laboratory practice recommendations, the performance of the research assay could not be controlled as strictly as in the case of routine laboratory tests. Thus, the differences in laboratory methods may also be partially responsible for the differences in results of the three studies. This implicates the need for standardization of the laboratory methods before wider clinical use of uPAR.

In our study, uPAR correlated significantly with the laboratory markers of liver injury (bilirubin, transaminanases). Previous reports in AP did not explore such correlations, however, similar relationships were observed in the critically ill [20]. Koch et al. [20] observed significant correlations between uPAR and the markers of renal function, not present in our study. However, in our patients, high uPAR was a predictor of the subsequent AKI. Moreover, we studied the associations between the demographic variables and uPAR and observed significantly higher concentrations in women. This is in line with previous reports, including the study of Nikkola et al. [36] in alcoholic AP, but also the studies regarding other groups of patients [38,39].

The main and the most important limitation of our study is the low number of patients with SAP. Moreover, for the above-mentioned reasons, the percentage of patients with MAP and MSAP differ from the general epidemiological data. Therefore, we cannot draw definitive conclusions. Nonetheless, our study suggests that circulating uPAR measured on admission to a secondary care hospital allows for the prediction of the complicated course of AP with moderate diagnostic accuracy, comparable to other inflammatory, coagulation or endothelial markers, including the ones widely available in the clinical practice (CRP, procalcitonin, and D-dimer). Thus, it is too early to advocate for the use of uPAR in the early assessment of AP severity. More studies are needed that would allow the evaluation of the robustness of uPAR diagnostic performance in other health care settings. Also, the lack of laboratory standardization of uPAR measurements must be taken into account before wider clinical use of the marker.

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Appendix

Table A1. The values of area under the receiver operating characteristic curve (AUC) with 95% confidence intervals (95% CI) for single laboratory markers proposed for the early assessment of severity of acute pancreatitis that were measured in the study and compared with uPAR. We provide AUC values estimated based on results obtained on the day of admission.

Laboratory Marker	AUC (95% CI); *p*-Value * for Prediction of		
	SAP	Organ Failure (MMSS ≥2 Points)	ICU Transfer or Death
IL-6	0.753 (0.590–0.917); $p = 0.002$	0.767 (0.578–0.956); $p = 0.006$	0.781 (0.610–0.953); $p = 0.001$
CRP	0.647 (0.412–0.882); $p = 0.2$	0.592 (0.373–0.810); $p = 0.4$	0.675 (0.425–0.925); $p = 0.2$
Procalcitonin	0.669 (0.378–0.961); $p = 0.3$	0.870 (0.729–1.000); $p < 0.001$	0.844 (0.627–1.000); $p = 0.002$
D-dimer	0.605 (0.377–0.901); $p = 0.4$	0.674 (0.485–0.863); $p = 0.07$	0.746 (0.582–0.909); $p = 0.003$
sFlt-1	0.770 (0.546–0.993); $p = 0.018$	0.921 (0.825–1.000); $p < 0.001$	0.758 (0.495–1.000); $p = 0.054$

* *p*-value in comparison with AUC = 0.5.

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Artykuł nr 3

Witold Kolber, Beata Kuśnierz-Cabala , Małgorzata Maraj, Małgorzata Kielar, Paulina Mazur , Barbara Maziarz, Paulina Dumnicka

Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure.

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Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure

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Abstract: B a c k g r o u n d: In early phase of acute pancreatitis (AP), systemic inflammatory response syndrome may lead to organ failure. The severe form of AP is associated with high mortality that may be prevented by timely diagnosis and treatment of the predicted severe cases. Serum interleukin 6 (IL-6) and urokinase-type plasminogen activator receptor (uPAR) have been proposed as accurate early markers of severe AP. The aim of the study was to assess whether widely available blood count indexes: neutrophil to lymphocyte (NLR), lymphocyte to monocyte (LMR) and platelet to lymphocyte ratios correlate with IL-6 and uPAR and may be utilized to predict organ complications at the early phase of AP.
M e t h o d s: The study included 95 adult patients with AP treated at the Surgical Ward Complex of Health Care Centers in Wadowice, Poland. Organ failure was diagnosed according to modified Marshall scoring system, as recommended by 2012 Atlanta classification. Blood samples for laboratory tests were collected on days 1, 2 and 3 following the onset of AP symptoms.

R e s u l t s: Patients with organ failure presented significantly lower LMR on day 1 and significantly higher NLR on days 2 and 3. Strong positive correlations between NLR and IL-6 and moderate correlations between NLR and uPAR were observed throughout the study. Day 2 and 3 NLR values significantly predicted organ failure at the early phase of AP.
C o n c l u s i o n s: Taking into account the wide availability of NLR, it may be considered as a surrogate of more expensive tests to help the early assessment of organ failure complicating AP.

Key words: urokinase-type plasminogen activator receptor, neutrophil-lymphocyte ratio, platelet--lymphocyte ratio, monocyte-lymphocyte ratio, persistent organ failure, interleukin 6.

Introduction

Acute pancreatitis (AP) is a relatively common acute digestive tract disorder, which in nearly 80% of patients progresses mildly and without complications (mild acute pancreatitis, MAP), but in about 20% of cases it develops into the severe form (severe acute pancreatitis, SAP) associated with nearly 20% mortality, reaching 50–80% in elderly patients or those with comorbidities such as chronic kidney disease, cardiovascular, autoimmunological disease or diabetes [1].

The medical advancement in imaging techniques witnessed in the last decades and the widespread availability of contrast-enhanced computed tomography, ultrasonography, and magnetic resonance have significantly facilitated the diagnostic process of AP and contributed to the timely detection of early necrotic changes [1]. According to the current Atlanta 2012 classification [2], imaging findings, beside typical symptoms and over threefold increase in pancreatic enzymes activity (amylase, lipase) are included in the diagnostic criteria for AP. The severity of AP in the revised 2012 Atlanta classifications was based on organ failure and local and systemic complications [2]. Notably, vital organ (cardiovascular, pulmonary and renal) failure persistent over 48 hours has been underscored as the main cause of early mortality in AP [2].

A significant progress has also been observed in the understanding of AP pathomechanism, in consequence of which a range of diagnostic tools for early SAP prediction has been proposed. The evaluation of laboratory markers has led to the development of prognostic scores such as Ranson, Glasgow, APACHE II or BISAP. Moreover, single biomarkers have emerged, which may help in timely prognosis of severe course of AP [1, 3–6].

A continuous and consecutive clinical observation of AP patients has highlighted the importance of the "therapeutic window" estimated at the first 48 hours from AP onset when an accurate diagnosis and immediate treatment can directly impact patients' survival. The most commonly used prognostic markers include acute phase proteins (C-reactive protein, albumin, fibrinogen), procalcitonin, total calcium

concentration, markers of renal function (urea, creatinine), coagulation parameters (D-dimers), white blood cell count and differential and recently also proinflammatory cytokine interleukin 6 (IL-6) [1, 3–6]. Recently, urokinase-type plasminogen activator receptor (uPAR) has also been suggested as an early prognostic marker of SAP [7].

Major prognostic scores — Ranson's or Glasgow-Imire's used in the first 48 hours from AP onset as well as BISAP used in the first 24 hours — include total white blood cell (WBC) count [8]. Other blood cell counts, i.e. absolute neutrophil, lymphocytes, monocytes or platelet counts and indexes based on the counts: neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) as well as lymphocyte to monocyte ratio (LMR) have not been as extensively used in day-to-day diagnostic process [1, 3–5, 9]. On the other hand, however, the review of literature on the prognostic significance of the decreased lymphocyte counts and increased NLR or PLR points to their promising diagnostic utility in SAP prognosis [3, 6, 8–12]. The study by Cho *et al.* [3] found that higher NLR or PLR values can indicate systemic inflammatory response syndrome (SIRS) and a risk of its developing into multi-organ dysfunction syndrome (MODS) [6, 13]. In 2017, Borges *et al.* [5] proposed Panc4, a new prognostic model, which comprises urea and creatinine, but also indexes: neutrophil/leukocytes, platelet/leukocytes ratio and NLR.

The aim of the study was to evaluate whether calculation of NLR, LMR and PLR on the first three days of AP can aid the prognosis of severe course of the disease, associated with vital organ failure at the early phase. Moreover, we assessed the correlations between the NLR, LMR and PLR indexes and more sophisticated laboratory markers of inflammation, including urokinase-type plasminogen activator receptor (uPAR) and IL-6 in serum over the first 72 hours from the onset of AP symptoms.

Materials and Methods

The study group included patients with AP diagnosis, treated at the Surgical Ward Complex of Health Care Centers in Wadowice from March 2014 until December 2015. The patients were included provided they were admitted within the first 24 hours from the onset of AP symptoms. The study group included solely adult patients with AP who provided their written voluntary consent to participate in the study. The exclusion criteria included liver, kidney disease, chronic pancreatitis or cancer at the moment of recruitment. AP diagnosis and the classification of AP severity were based on the revised 2012 Atlanta classification [2], according to which two out of the three listed criteria must be met: typical abdominal pain, at least a threefold increase in pancreatic enzymes' activity and imaging findings characteristic of AP (in computed tomography, ultrasonography or magnetic resonance imaging). Mild (MAP), moderately severe (MSAP) and severe (SAP) acute pancreatitis were distinguished based on the course of the disease.

Organ failure during the first week of AP was assessed according to the modified Marshall scoring system (MMSS) [2]. The Bioethics Committee of the Beskidy Medical Chamber approved the study protocol (approval number 2014/02/06/1 issued on 6 February 2014).

Blood samples for laboratory tests were collected from the patients thrice: within the first 24 hours from the onset of AP symptoms (day 1), and then on the following two days (day 2 and day 3). The routine tests (complete blood counts, serum amylase, alanine and aspartate transaminase, and lactate dehydrogenase activities, serum albumin, total protein, total calcium, glucose, urea, creatinine, bilirubin, procalcitonin and C-reactive protein concentrations, and plasma D-dimer concentrations) were conducted in the Diagnostic Laboratory in Wadowice. Complete blood counts were performed with automated hematologic analyzer Sysmex XN (Sysmex Corporation, Cobe, Japan). Based on the absolute counts of neutrophils, lymphocytes, monocytes and platelets, NLR, LMR, and PLR indexes were calculated. For the assessment of routine biochemical and immunochemical tests, Cobas E411 (Roche Diagnostics, Mannheim, Germany) and Vitros 5600 (Ortho Clinical Diagnostics, Raritan, NJ, USA) were used, whereas the coagulation tests were conducted with Coag XL (Diagno, Budapest, Hungary).

Moreover, the aliquots of the sera were used for the measurements of IL-6 and uPAR concentrations. IL-6 was measured in the Diagnostics Department of University Hospital in Krakow by automatized electrochemiluminescence assay (ECLIA) on the Cobas 8000 analyzer (Roche Diagnostics, Manheim, Germany). The measurements of uPAR were conducted at the Department of Diagnostics, Chair of Clinical Biochemistry at Jagiellonian University in Krakow using the Quantikine Human uPAR Immunoassay reagent kits (R&D Systems, Minneapolis, USA). The intraassay precision for the test was ≤7.5% and the interassay precision ≤5.6%, the minimum detectable dose of uPAR was 0.033 ng/mL, while the concentrations of uPAR in healthy volunteers were between 1.195 and 4.414 ng/mL as provided by the manufacturer of the kit.

Statistical analysis

Data on categories were reported as the number of patients (percentage of the appropriate group). As most of the quantitative variables were non-normally distributed (according to Shapiro-Wilk's test), we reported the median [lower; upper quartile], while the mean and standard deviation (SD) were provided for age. Contingency tables were analyzed with chi-squared test. The differences between the groups were assessed with Mann-Whitney's test. The correlations were assessed with Spearman's rank coefficient. The tests were two-tailed and the p-values ≤0.05

indicated statistically significant results. We used Statistica 12 (StatSoft, Tulsa, OK, USA) for computations.

Results

Comparison of NLR, LMR and PLR values between patients with and without organ failure

The study group included 95 patients: 65 (68%) men and 30 (32%) women. Mean (SD) age of the study group was 48 (17) years. During the first week of AP, organ failure with ≥2 points in MMSS was diagnosed in nine patients. The baseline clinical characteristics of the study patients who developed organ failure and those without organ failure were presented in Table 1.

Table 1. Clinical characteristics of the study group according to the score in modified Marshall scoring system (MMSS).

Characteristic	MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
Male sex, n (%)	7 (78)	58 (67)	0.5
Mean age (SD), years	57 (20)	47 (16)	0.2
Pre-existing comorbidities, n (%)	4 (44)	38 (44)	0.9
Cardiac diseases, n (%)	4 (44)	26 (30)	0.4
Diabetes, n (%)	1 (11)	7 (8)	0.8
Etiology:			
Biliary, n (%)	3 (33)	24 (28)	0.9
Alcoholic, n (%)	3 (33)	26 (30)	
Hypertriglyceridemia, n (%)	0	5 (6)	
Other/idiopathic, n (%)	3 (33)	31 (36)	
Pancreatic or peripancreatic necrosis, n (%)	2 (22)	10 (12)	0.4
Median Ranson score [Q1; Q3], points	6 [5; 7]	3 [2; 4]	<0.001
Median BISAP [Q1; Q3], points	3 [2; 3]	2 [2; 2]	0.022
Median duration of hospital stay [Q1; Q3], days	15 [10; 27]	12 [8; 15]	0.5
SIRS in first 24 hours, n (%)	8 (89)	66 (77)	0.4
Early/late mortality, n (%)	1 (11)/2 (22)	0/1 (1)	<0.001

Table 1. Cont.

Characteristic		MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
Therapeutic ERCP, n (%)		2 (22)	3 (3)	0.016
Surgery, n (%)		4 (44)	4 (5)	<0.001
Transfer to ICU, n (%)		5 (56)	2 (2)	<0.001
Final severity	MAP, n (%)	0	29 (34)	<0.001
	MSAP, n (%)	4 (44)	54 (63)	
	SAP, n (%)	5 (56)	3 (3)	

ERCP — endoscopic retrograde cholangiopancreatography; ICU — intensive care unit; MAP — mild acute pancreatitis; MSAP — moderately severe acute pancreatitis; n — number of patients; SAP — severe acute pancreatitis; SIRS — systemic inflammatory response syndrome; SD — standard deviation; Q1 — lower quartile; Q3 — upper quartile.

On the first day of observation, the WBC counts of patients with AP were in the wide range from $5.2 \times 10^3/\mu L$ to $32.8 \times 10^3/\mu L$. There were no statistically significant differences in the total WBC counts between patients with MMSS ≥2 points and those without organ failure (Table 2). The average neutrophil counts and percentages were higher in patients with high MMSS, while the lymphocyte counts were lower, but the differences were also statistically non-significant (Table 2). The median monocyte count in high MMSS group was twice as high as in low MMSS patients, however, in both groups the variability of monocyte counts was high and thus the difference was non-significant (Table 2). The platelet counts in both groups were comparable (Table 2). The values of NLR and PLR observed on day 1 of the study did not differ significantly between the groups, however, LMR was significantly lower among the patients with MMSS ≥2 points.

Table 2. The results of laboratory tests in the study group according to the score in modified Marshall scoring system (MMSS). Data are shown as median [Q1; Q3].

Characteristic	MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
WBC, $\times 10^3/\mu L$	13.7 [9.7; 21.9]	12.4 [10.3; 15.6]	0.3
Neutrophils, $\times 10^3/\mu L$	15.4 [8.6; 24.2]	10.3 [6.9; 14.7]	0.3
Neutrophils, %	89.5 [78.3; 92.5]	83.5 [77.9; 86.7]	0.2
Lymphocytes, $\times 10^3/\mu L$	0.77 [0.46; 1.38]	1.16 [0.66; 1.61]	0.3
Lymphocytes, %	2.85 [2.6; 14.6]	9.3 [4.9; 13.7]	0.2

Table 2. Cont.

Characteristic	MMSS ≥2 (n = 9)	MMSS <2 (n = 86)	p-value
NLR	31.17 [16.77; 34.75]	8.61 [5.59; 18.49]	0.2
Monocytes, $\times 10^3/\mu L$	1.71 [0.57; 2.0]	0.84 [0.62; 1.07]	0.3
Monocytes, %	5.2 [4.2; 9.8]	6.2 [5.3; 7.9]	0.6
LMR	0.47 [0.28; 0.57]	1.44 [0.78; 1.95]	0.033
Hemoglobin, g/dL	14.9 [14.1; 15.4]	15.4 [14; 16.7]	0.6
RBC, $\times 10^6/\mu L$	4.68 [4.56; 4.90]	4.90 [4.46; 5.29]	0.5
Hematocrit, %	43 [41.7; 46]	43.7 [40.6; 46.5]	0.9
Platelets, $\times 10^3/\mu L$	189 [165; 278]	204 [168.5; 261]	0.9
PLR	278.3 [138.4; 969.6]	171.5 [111.2; 253.2]	0.3
Serum amylase, U/L	492 [331; 1996]	473 [171; 1812]	0.7
Albumin, g/L	33 [26; 26]	37 [33; 41]	0.1
Total protein, g/L	69 [61; 77]	65 [63; 77]	0.8
Total calcium, mmol/L	2.05 [1.85; 2.28]	2.14 [1.98; 2.28]	0.4
Glucose, mmol/L	7.72 [6.94; 9.16]	7.44 [6.16; 9.0]	0.5
Urea, mmol/L	8.66 [5.83; 13]	4.33 [3.33; 5.83]	<0.001
Creatinine, µmol/L	166.1 [86.6; 184.7]	68.1 [58.3; 84.8]	<0.001
Bilirubin, µmol/L	30.9 [19.8; 49.5]	24.9 [13.8; 39.6]	0.3
AST, U/L	118 [82; 166]	91 [46; 202]	0.2
ALT, U/L	77 [54; 92]	107 [49; 243]	0.8
LDH, U/L	854 [698; 1281]	619 [498.5; 813.5]	0.07
CRP, mg/L	36.6 [14,8; 338]	25.4 [8.7; 178]	0.4
D-dimer, mg/L	3076.6 [1499.4; 4888.6]	1788.4 [883.7; 2917]	0.1
Procalcitonin, ng/mL	1.68 [0.29; 17]	0.14 [0.05; 0.36]	0.001
uPAR, ng/mL	6.73 [3.98; 7.76]	3.80 [2.90; 4.92]	0.010
Interleukin 6, pg/mL	724.7 [62.7; 1713]	72.75 [23.93; 145.5]	0.013

ALT — alanine aminotransferase; AST — aspartate aminotransferase; CRP — C-reactive protein; IL-6 — interleukin 6; LDH — lactate dehydrogenase; LMR — lymphocyte to monocyte ratio; NLR — neutrophil to lymphocyte ratio; PLR — platelet to lymphocyte ratio; RBC — red blood cells; uPAR — urokinase-type plasminogen activator receptor; WBC — white blood cells.

On study day 2 and 3, lymphocyte counts differed significantly between patients with MMSS ≥2 points and <2 points (Fig. 1). The differences between the groups in WBC and neutrophil counts became significant on day 3 of the study (Fig. 1). On days 2 and 3, the difference in NLR between the patients with MMSS above

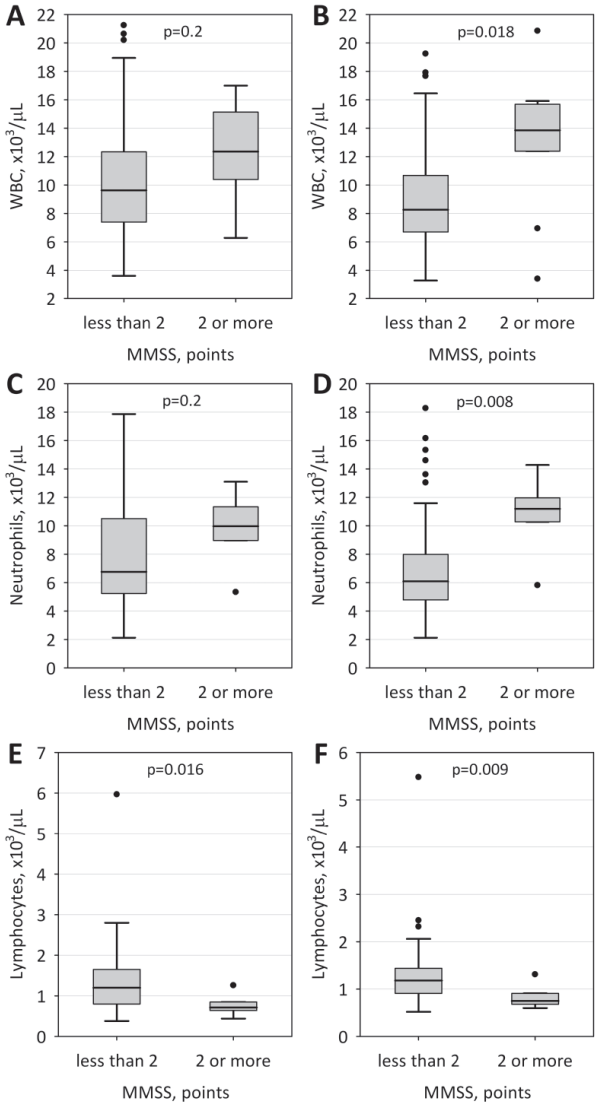

Fig. 1. WBC (A, B), neutrophil (C, D) and lymphocyte (E, F) counts on day 2 (A, C, E) and day 3 (B, D, F) of the study among patients with acute pancreatitis with and without organ failure associated with two or more points in modified Marshall scoring system (MMSS). Data are shown as median, interquartile range (box), non-outlier range (whiskers) and outliers (dots).

and below 2 points was highly significant (Fig. 2). As on day 1, we observed no differences between the groups in day 2 and 3 monocyte and platelet counts ($p > 0.4$ in all cases). Moreover, day 2 and 3 LMR and PLR values did not differ between the groups (Fig. 2).

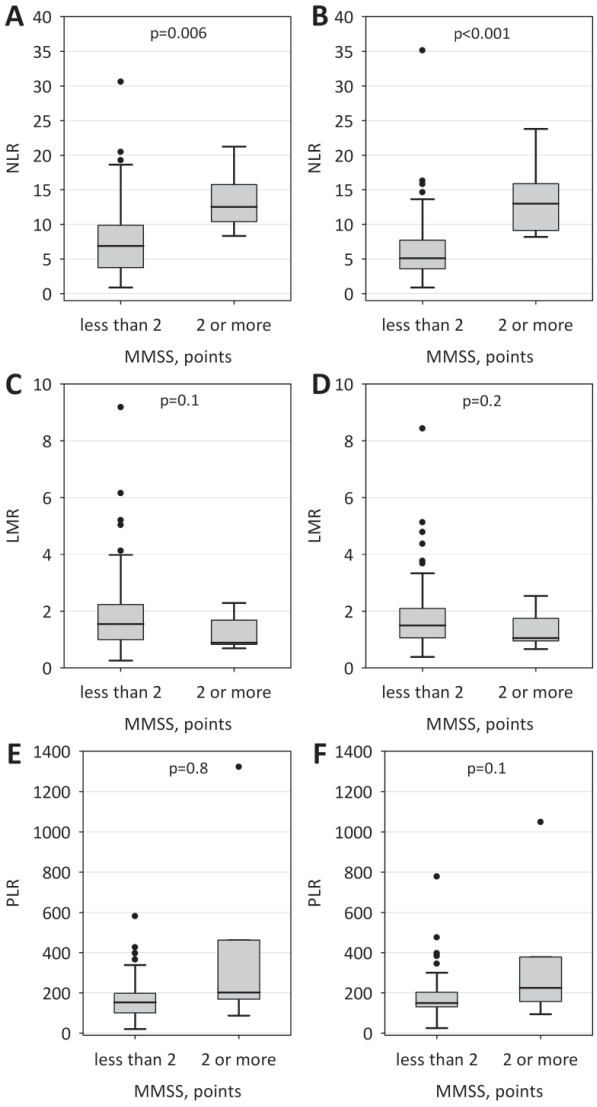

Fig. 2. NLR (A, B), LMR (C, D) and PLR (E, F) indexes on day 2 (A, C, E) and day 3 (B, D, F) of the study among patients with acute pancreatitis with and without organ failure associated with two or more points in modified Marshall scoring system (MMSS). Data are shown as median, interquartile range (box), non-outlier range (whiskers) and outliers (dots).

In logistic regression analysis, NLR values assessed on day 2 and 3, as well as WBC, neutrophil and lymphocyte count on day 3 of the study significantly predicted organ failure in the early phase of AP (Table 3).

Table 3. Statistically significant predictors of organ failure in the first week of acute pancreatitis associated with two or more points in modified Marshall scoring system. Odds ratios (OR) in simple logistic regression analysis per 1 unit increase in the value of predictor variable are given with 95% confidence intervals (CI).

Predictor variable	Day 2		Day 3	
	OR (95% CI)	p-value	OR (95% CI)	p-value
WBC, per 1 $\times 10^3/\mu L$	–	–	1.28 (1.07–1.54)	0.007
Neutrophils, per 1 $\times 10^3/\mu L$	–	–	1.30 (1.04–1.62)	0.017
Lymphocytes, per 1 $\times 10^3/\mu L$	–	–	0.01 (0.0002–0.65)	0.028
NLR, per 1	1.15 (1.01–1.31)	0.028	1.18 (1.03–1.35)	0.016

Comparison of other laboratory markers between patients with and without organ failure

Among studied laboratory markers, urea, creatinine, procalcitonin, uPAR and IL-6 concentrations were significantly higher among patients with organ failure (MMSS ≥2 points) than among those without the complication (Table 2). These differences remained statistically significant throughout the study. Moreover, on day 2 and 3, patients with MMSS ≥2 points presented higher concentrations of bilirubin (median 38.9 versus 18.6 μmol/L on day 2; $p = 0.029$, and 35.3 versus 15.1 μmol/L on day 3; $p = 0.019$, respectively), and higher amylase activity in sera (median 590 versus 141 U/L on day 2; $p = 0.032$, and 184 versus 76 U/L on day 3; $p = 0.041$, respectively).

Correlations of CBC values and NLR, LMR and PLR indexes with IL-6, uPAR and other laboratory markers

In the whole studied group of patients at the early phase of AP, we observed significant correlations between the studied CBC values as well as the NLR, LMR, and PLR indexes, and the results of laboratory tests associated with organ failure and inflammation (Table 4).

WBC (on day 3), neutrophil count (on days 1 and 3), and NLR (on days 2 and 3) correlated positively with urea concentrations, while negative correlations were observed between urea and lymphocyte count (on day 2) and LMR (on day 2) (Table 4). Monocyte count correlated positively with serum creatinine (throughout

the study), and there was a negative correlation between platelet count and creatinine on day 3. Serum bilirubin correlated positively with NLR (on day 2), and negatively with lymphocyte and platelet counts (on days 2 and 3). Serum lactate dehydrogenase activity on days 2 and 3 was positively correlated with WBC, neutrophil, monocyte counts and NLR and negatively with lymphocyte, platelet counts and LMR (Table 4).

Table 4. Statistically significant correlations of white blood cell counts, differential counts, neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR) and platelet to lymphocyte ratio (PLR) with selected laboratory markers during early stage of acute pancreatitis.

Variable	Day 1		Day 2		Day 3	
	R	p-value	R	p-value	R	p-value
White blood cell count						
Urea	–	–	–	–	0.24	0.02
LDH	–	–	–	–	0.30	0.01
CRP	0.21	0.04	0.40	<0.001	0.50	<0.001
Procalcitonin	0.29	0.01	0.37	<0.001	0.52	<0.001
D-Dimer	–	–	0.34	0.001	0.46	<0.001
IL-6	0.37	<0.001	0.50	<0.001	0.59	<0.001
Ranson score	–	–	0.41	<0.001	–	–
Absolute neutrophil count						
Urea	0.42	0.004	–	–	0.30	0.006
LDH	–	–	0.32	0.006	0.27	0.02
CRP	–	–	0.48	<0.001	0.55	<0.001
Procalcitonin	0.33	0.03	0.52	<0.001	0.61	<0.001
D-Dimer	–	–	0.45	<0.001	0.48	<0.001
uPAR	–	–	–	–	0.26	0.03
IL-6	0.42	0.006	0.63	<0.001	0.63	<0.001
Ranson score	–	–	0.45	<0.001	–	–
NLR						
Urea	–	–	0.37	0.001	0.33	0.003
Bilirubin	–	–	0.23	0.04	–	–
LDH	–	–	0.39	<0.001	0.44	<0.001
CRP	–	–	0.51	<0.001	0.57	<0.001
Procalcitonin	0.51	<0.001	0.61	<0.001	0.68	<0.001
D-Dimer	–	–	0.40	<0.001	0.47	<0.001

Table 4. Cont.

Variable	Day 1		Day 2		Day 3	
	R	p-value	R	p-value	R	p-value
uPAR	0.36	0.03	0.34	0.002	0.40	<0.001
IL-6	0.53	<0.001	0.53	<0.001	0.64	<0.001
Ranson score	–	–	0.29	0.008	–	–
Absolute lymphocytes count						
Urea	–	–	−0.28	0.01	–	–
Bilirubin	–	–	−0.37	<0.001	−0.26	0.02
LDH	–	–	−0.25	0.03	−0.37	0.001
CRP	–	–	−0.28	0.01	−0.26	0.01
Procalcitonin	−0.40	0.007	−0.38	<0.001	−0.41	<0.001
uPAR	−0.34	0.03	−0.42	<0.001	−0.38	<0.001
IL-6	−0.29	0.06	−0.27	0.006	−0.29	0.009
Absolute monocyte count						
Creatinine	0.37	0.01	0.24	0.03	0.29	0.01
LDH	–	–	–	–	0.40	<0.001
CRP	–	–	–	–	0.45	<0.001
Procalcitonin	0.38	0.01	–	–	0.41	<0.001
D-Dimer	–	–	–	–	0.46	<0.001
uPAR	–	–	–	–	0.27	0.02
IL-6	–	–	–	–	0.53	<0.001
LMR						
Urea	–	–	−0.26	0.02	–	–
LDH	–	–	−0.28	0.01	−0.28	0.02
CRP	–	–	−0.33	0.003	−0.29	0.01
Procalcitonin	−0.52	<0.001	−0.33	0.004	−0.37	0.001
D-Dimer	–	–	−0.32	0.005	–	–
uPAR	−0.35	0.04	−0.26	0.02	–	–
IL-6	−0.50	0.001	−0.30	0.009	−0.32	0.005
Platelet count						
Bilirubin	–	–	−0.46	<0.001	−0.41	<0.001
LDH	–	–	−0.32	0.006	–	–

Table 4. Cont.

Variable	Day 1		Day 2		Day 3	
	R	p-value	R	p-value	R	p-value
Creatinine	–	–	–	–	–0.23	0.03
CRP	–0.20	0.06	–	–	–	–
PLR						
Procalcitonin	–	–	–	–	0.24	0.03
uPAR	–	–	0.23	0.04	–	–
IL-6	–	–	–	–	0.25	0.02

ALT — alanine aminotransferase; AST — aspartate; CRP — C-reactive protein; IL-6 — interleukin 6; LDH — lactate dehydrogenase; LMR — lymphocyte to monocyte ratio; NLR — neutrophil to lymphocyte ratio; PLR — platelet to lymphocyte ratio; uPAR — urokinase-type plasminogen activator receptor; WBC — white blood cells.

The studied laboratory markers of inflammation correlated positively with WBC, neutrophil count, NLR, monocyte count, and PLR, and negatively with lymphocyte count, and LMR (Table 4). Total WBC counts and NLR values were significantly associated with CRP, procalcitonin and IL-6 throughout the study, and with D-dimer on days 2 and 3. Moreover, NLR positively correlated with serum uPAR during the entire observation period. Neutrophil counts significantly correlated with procalcitonin and IL-6 throughout the study, with CRP and D-dimer on days 2 and 3 and with uPAR on day 3. Lymphocyte counts were negatively associated with uPAR, IL-6 and procalcitonin on days 1 to 3, and with CRP on days 2 and 3. Only day 3 monocyte counts correlated with the studied inflammatory markers, except for correlation with procalcitonin on day 1, while more significant correlations were observed for LMR (Table 4). In case of platelet counts, only a weak negative correlation with CRP was observed on day 1, while PLR positively correlated with uPAR (on day 2), procalcitonin and IL-6 (on day 3) (Table 4).

Moreover, significant correlations were observed between Ranson's score and WBC, neutrophil, count and NLR (Table 4).

Discussion

The increase in peripheral blood neutrophil counts and the decrease in lymphocyte counts is commonly observed in patients with SIRS developing in the course of various diseases, including AP, severe septic complications, bacteremia, as a consequence of extensive surgical injury or after a cardiothoracic surgery [1, 8, 11, 13, 14]. It has been proposed previously that monitoring the values of NLR can be more useful than total WBC counts in differentiating between the mild and severe AP [14]. In the course

of AP neutrophils propagate inflammation and tissue injury via the activation of a wide range of mediators and inflammatory markers i.e. proinflammatory cytokines (IL-6, IL-8, tumor necrosis factor a), proteolytic enzymes (elastase, myeloperoxidase, collagenase) and reactive oxygen species. In turn, lymphopenia in the first 24 hours can be an independent marker of progression of inflammation, necrosis and septic complications and it remains a negative prognostic factor in this group of patients [1, 8, 11]. Shen *et al.* [11] revealed that reduced lymphocyte count within 48 hours from AP onset reflects the dysfunction of the immune system and might be an early and effective predictor of pancreatic necrosis [11].

The present study showed that in patients with vital organ failure in the early phase of AP (MMSS ≥2 points in the first week of AP), lower absolute lymphocyte counts were observed on days 2 and 3 from the onset of AP symptoms in comparison to patients without organ failure. The lymphocyte counts observed in patients with organ failure were, in fact, below the lower reference limit of 1.0 to 5.0 $\times 10^3/\mu L$ already on day 1 of AP. Simultaneously, in the whole studied group, we observed absolute neutrophilia and increased NLR index as compared to the previously reported reference values (1.8 to 8.0 $\times 10^3/\mu L$ and 0.78 to 3.53, respectively) [15, 16]. NLR on days 2 and 3 from AP onset significantly predicted vital organ failure developing in the first week of the disease.

The widely available automated 5-diff hematological analyzers can provide 28 diagnostic parameters which in the case of WBC differentiate their 5 main populations i.e. neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The wide panel of red blood cell indexes may be used for the evaluation of anemia. The extended flagging system enables the identification of almost 30 various abnormalities regarding the CBC result [16]. The recommendations established in 1992 by the National Committee for Clinical Laboratory Standards [currently functioning under the name of Clinical and Laboratory Standards Institute (CLSI)] advocated for the use of absolute cell counts instead of percentages of white blood cells subpopulations, in consequence of wide availability of automated blood cell counters. This was a significant shift in the interpretation of CBC results that also enabled the use of indexes such as NLR, LMR, or PLR in the routine medical diagnostics [16]. At present, the values of NLR, PLR, and LMR indexes can be easily obtained following ordering an automated CBC with WBC differential. Their availability in everyday clinical practice depends solely on the laboratory's decision to include them in the diagnostic panel by implementing the simple formulas for their mathematical computation. This wide availability encouraged us to analyze the indexes in association with more sophisticated laboratory markers such as IL-6 and uPAR with respect to early prediction of organ failure in AP.

We observed significantly lower LMR values on day 1. The result is in line with the study by Li *et al.* [9], which showed that LMR index has a minor prognostic

value in SAP prediction. LMR index, however, has not been yet carefully studied in AP, and the most abundant research on its prognostic value concerns primarily colorectal cancer [17], pancreatic cancer [18–20], hematological malignancies [21] and breast cancer [22]. The mentioned studies pointed to correlations between the LMR values and the degree of systemic inflammation, as well as progression-free and overall survival. The significantly higher NLR values on days 2 and 3 in patients with complicated course of AP are also in accordance with the previous observations [3, 8, 12, 14]. The work by Cho *et al.* [3] as the only one highlights clearly higher NLR and PLR indexes values in patients with biliary etiology of SAP [3]. In 2011, Azab *et al.* [14] found that the NLR >4.7 allows for an accurate prognosis of SAP and the necessity to transfer patients to intensive care unit [6, 12, 14]. Our study did not confirm the observations regarding PLR, including those of Kaplan *et al.* [6] who showed significantly higher values of PLR index in patients with severe or complicated course of AP [6].

In our study, NLR values strongly positively correlated with procalcitonin and IL-6 concentrations and also positively correlated with serum uPAR during the whole observation time. Moreover, it correlated with several markers of organ dysfunction (urea, bilirubin, lactate dehydrogenase) and the values of Ranson's score. A statistically significant inverse correlation was observed between LMR and both procalcitonin and IL-6 on all days of the study as well as with uPAR on days 1 and 2. Our previous studies on diagnostic usefulness of IL-6 in the early phase of AP demonstrated that it is useful for prediction of severe course of AP [23]. IL-6 is one of the cytokines which first reaches the inflammation site and is produced by a wide array of cells: monocytes, T-cells, B-cells, neutrophils, fibroblasts and also pancreatic acinar cells [23, 24]. In prediction of SAP, it has been shown to complement the SIRS criteria [24]. As IL-6 is not available in most routine diagnostic laboratories, NLR may be considered as a surrogate marker in the early phase of AP.

uPAR, in turn, is a glycoprotein expressed on various cells, including monocytes, macrophages, neutrophils, endothelial cells and some cancer cells [25]. uPAR and its urokinase-type plasminogen activator ligand play an important role in the immunological response linked with migration, adhesion, angiogenesis, fibrinolysis, and cell proliferation [26]. The increase in serum concentrations of the soluble form of the receptor has been linked with the activation of the immunological system and reflects the severity of inflammatory diseases. In the present study, we observed significantly higher concentrations of uPAR among the patients with vital organ failure starting from day 1 from the onset of AP symptoms. Significant positive correlations were observed between uPAR and NLR as well as negative correlations with absolute lymphocyte counts on each study day. Moreover, LMR negatively correlated with uPAR on two first days of the study. Previously, uPAR has been associated with the severity of AP as well as other acute states [7, 27], thus its correlation with NLR

can additionally justify the need for the evaluation of NLR values in the daily clinical practice.

Conclusions

Since the computation of indexes: NLR, LMR and PLR is easily attainable based on routine CBC with WBC differential, incorporating them into routine diagnostics has been suggested. Constantly increased values of NLR observed in the first three days following the onset of AP in the patients who developed early organ failure and the correlations of NLR with more sophisticated inflammatory markers (including uPAR and IL-6) point to the potential usefulness of this index in the early prognosis of the severe course of AP.

Conflict of interest

None declared.

Acknowledgements

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23. *Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., et al.*: Does the automatic measurement of interleukin 6 allow for predict of complications during the first 48 h of acute pancreatitis? Int J Mol Sci. 2018; 19: 1820. doi. 10.3390/ijms19061820.
24. *Jain S., Midha S., Mahapatra S.J., Gupta S., Sharma M.K., Nayak B., et al.*: Intereukin-6 significantly improves predictive value of systemic inflammatory response syndrome for predicting severe acute pancreatitis. Pancreatology. 2018; 1–7. doi. 10.1016/j.pan.2018.05.002.

25. *Kolber W., Kuśnierz-Cabala B., Dumnicka P., Maraj M., Mazur-Laskowska M., Pędziwiatr M., et al.*: Serum urokinase-type plasminogen activator receptor does not outperform C-reactive protein and procalcitonin as an early marker of severity of acute pancreatitis. J Clin Med. 2018; 7: 305. doi: 10.3390/jcm7100305.
26. *Khater W.S., Salah-Eldeen N.N., Khater M.S., Saleh A.N.*: Role of uPAR and lactatic acid in diagnosing sepsis and predicting mortality in elderly patients. Eur J Micr Immunol. 2016; 3: 178–185. doi: 10.1556/1886.2016.00011.
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Oświadczenia współautorów artykułów określające indywidualny wkład współautorów w powstawaniu prac

Kraków, dnia 14 styczeń 2019 r.

mgr Małgorzata Maraj

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.
2. Kolber W., Kuśnierz-Cabala B., Dumnicka P., Maraj M., Mazur-Laskowska M., Pędziwiatr M., Ceranowicz P.: Serum urokinase–type plasminogen activator receptor does nor outperform C-reactive protein and procalcitonin as an early marker of severity of acute pancreatitis. *J Clin Med.* 2018; 7 (305): doi.: 10.3390/jcm7100305.
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oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
pomoc w przygotowaniu oznaczeń, tłumaczenie tekstu na język angielski, korekta językowa, tworzenie bazy piśmiennictwa do prac

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Witolda Kolbera przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

Małgorzata Maraj

.....

(podpis współautora)

Kraków, dnia 6 lutego 2019 r.

dr hab. n. med. Michał Pędziwiatr, prof. UJ

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.
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udział w opracowaniu materiału klinicznego, udział w tworzeniu koncepcji badawczej, udział w poprawie manuskryptów i odpowiedziach do recenznetów

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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Michał Pędziwiatr

(podpis współautora)

Kraków, dnia 4 lutego 2019 r.

Dr n. med. Paulina Dumnicka

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.
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oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
wykonanie obliczeń statystycznych, opracowanie graficzne danych, merytoryczna dyskusja wyników, tłumaczenie tekstu na język angielski oraz udział merytoryczny w przygotowaniu odpowiedzi na recenzje

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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P. Dumnicka

.....

(podpis współautora)

Kraków, dnia 4 lutego 2019 r.

Mgr Paulina Mazur

.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.:

1. Kolber W., Kuśnierz-Cabala B., Maraj M., Kielar M., Mazur P., Maziarz B., Dumnicka P.: Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure. *Folia Med. Crac.* 2018; 4: 57-74.

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przygotowanie materiału do badań, pomoc w opracowaniu wyników badań laboratoryjnych

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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Paulina Mazur
.....
(podpis współautora)

Kraków, dnia 7 styczeń 2019 r.

Dr n. med. Małgorzata Mazur-Laskowska

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.
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wykonanie oznaczeń biochemicznych i immunochemicznych, opracowanie bazy danych

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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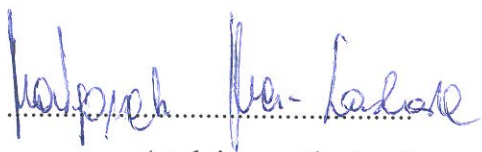

.....

(podpis współautora)

Kraków, dnia 14 styczeń 2019 r.

Prof. dr hab. n. med. Beata Kuśnierz-Cabala

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.
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udział merytoryczny w stworzeniu i opracowaniu koncepcji manuskryptów, wykonanie badań laboratoryjnych, udział w opracowaniu bazy danych i dyskusja wyników, a także udział w przygotowaniu, publikacji oraz korespondencji z redakcją czasopism

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Witolda Kolbera przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

B. Kuśnierz-Cabala

.....

(podpis współautora)

Kraków, dnia 14 styczeń 2019 r.

Mgr Małgorzata Kielar

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Kuśnierz-Cabala B., Maraj M., Kielar M., Mazur P., Maziarz B., Dumnicka P.: Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure. *Folia Med. Crac.* 2018; 4: 57-74.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:
pomoc w wykonaniu badań laboratoryjnych i przygotowaniu bazy danych

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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Kielar Małgorzata

.....

(podpis współautora)

Kraków, dnia 7 styczeń 2019 r.

Prof. dr hab. n. med. Marek Kuźniewski

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.

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udział w opracowaniu klinicznej strony zagadnienia, dyskusja kliniczna i udział w przygotowaniu manuskryptu oraz odpowiedzi dla recenzentów

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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M. Kuźniewski

.....

(podpis współautora)

Kraków, dnia 4 lutego 2019 r.

Dr n. med. Mateusz Sporek

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.

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udział w zebraniu materiału klinicznego i pomoc w przygotowaniu bazy danych pacjentów

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek Witolda Kolbera jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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Mateusz Sporek

.....

(podpis współautora)

Kraków, dnia 14 styczeń 2019 r.

Dr n. med. Barbara Maziarz

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor prac pt.:

1. Kolber W., Dumnicka P., Maraj M., Kuśnierz-Cabala B., Ceranowicz P., Pędziwiatr M., Maziarz B., Mazur-Laskowska M., Kuźniewski M., Sporek M., Walocha J.: Does the automatic measurement of interleukin 6 allow for prediction of complications during the first 48 h of acute pancreatitis? *Int J Mol Sci.* 2018; 19 (1820): doi.: 10.3390/ijms19061820.
2. Kolber W., Kuśnierz-Cabala B., Maraj M., Kielar M., Mazur P., Maziarz B., Dumnicka P.: Neutrophil to lymphocyte ratio at the early phase of acute pancreatitis correlates with serum urokinase-type plasminogen activator receptor and interleukin 6 and predicts organ failure. *Folia Med. Crac.* 2018; 4: 57-74.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to:

przygotowanie materiału do badań oraz udział w przeprowadzeniu oznaczeń laboratoryjnych

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Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Witolda Kolbera przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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(podpis współautora)

Kraków, dnia 7 styczeń 2019 r.

Prof. dr hab. n. med. Piotr Ceranowicz

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

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Piotr Ceranowicz

.....

(podpis współautora)

Spis skrótów

Skrót	Nazwa angielska	Nazwa polska
AKI	*Acute kindney injury*	Ostre uszkodzenie nerek
Ang-2	*Angiopoietin-2*	Angiopoetyna-2
AP	*Acute pancreatitis*	Ostre zapalenie trzustki
APACHE II	*Acute Physiology and Chronic Health Evaluation II*	-
ARDS	*Acute respiratory distress syndrome*	Zespół ostrej niewydolności oddechowej
AST; ALT	*Aspartate and alanine aminotransferase*	Aminotransferazy (asparaginianowa i alaninowa)
BISAP	*Bedside Index of Severity in AP*	-
BUN	*Blood urea nitrogen*	Azot mocznika
CARS	*Compensatory anti-inflammatory response syndrome*	Kompensacyjny zespół odpowiedzi przeciwzapalnej
CRP	*C-reactive protein*	Białko C-reaktywne
ECLIA	*Immunochemiluminometric assay*	Test immunochemiluminescencyjny
ERCP	*Endoscopic retrograde cholangiopancreatogrphy*	Endoskopowa wsteczna cholangiopankreatografia
IL-6	*Interleukin 6*	Interleukina 6
KDIGO	*Kidney Disease Improving Global Outcome*	-
KIM-1	*Kidney injury molekule-1*	Cząsteczka uszkodzenia nerek-1
LDH	*Lactate dehydrogenase*	Dehydrogenaza mleczanowa
L-FABP	*Liver-type fatty acid binding protein*	Wątrobowy typ białka wiążącego kwasy tłuszczowe
LMR	*Lymphocyte to monocyte ratio*	Wskaźnik limfocytarno-monocytarny
MAP	*Mild acute pancreatitis*	Łagodna postać ostrego zapalenia trzustki
MMSS	*Modified Marshall Scoring System*	Zmodyfikowana skala Marshalla
MODS	*Multiple Organ Dysfunction*	Zespół niewydolności

	Syndrome	wielonarządowej
MSAP	*Moderately severe acute pancreatitis*	Średnio-ciężka postać ostrego zapalenia trzustki
NEU	*Neutrophils*	Neutrofile
NLR	*Neutrophil to lymphocyte ratio*	Wskaźnik neutrofilowo-limfocytarny
OZT	*Aute pancreatitis*	Ostre zapalenie trzustki
PCT	*Procalcitonin*	Prokalcytonina
PLR	*Platelet to lymphocyte ratio*	Wskaźnik płytkowo-limfocytarny
SAP	*Severe acute pancreatitis*	Ciężka postać ostrego zapalenia trzustki
SAPS	*Sequential Organ Failure Assessment*	-
sFlt-1	*Soluble fms-like tyrosine kinase 1*	fms-podobna kinaza tyrozynowa 1
SIRS	*Systemic Inflammatory Response Syndrome*	Zespół uogólnionej odpowiedzi zapalnej
TNF	*Tumor necrosis factor*	Czynnik martwicy nowotworów
uPAR	*Urokinase-type plasmingen activator receptor*	Receptor aktywatora plazminogenu typu urokinazowego
WBC	*White blood cells*	Liczba leukocytów

Załącznik nr 1.

Zakresy wartości referencyjnych dla badań laboratoryjnych wykorzystywanych w pracy.

Nazwa badania	Przedział referencyjny
Morfologia krwi obwodowej	
Liczba erytrocytów, x 10^6/μL	K: 3,5 – 5,5/ M: 4,5 – 6,5
Hemoglobina, g/dL	K: 11,0 – 15,0/ M: 12,0 – 17,0
Hematokryt, %	K: 37,0 – 47,0/ M: 40,0 – 54,0
Liczba leukocytów, x 10^3/ μL	K/M: 4,0 – 10,0
Liczba neutrofilów, x 10^3/μL; (%)	K/M: 1,8 – 8,0 (58,0 – 66,0)
Liczba limfocytów, x 10^3/μL; (%)	K/M: 1,0- 5,0 (20-45)
Liczba monocytów, x 10^3/ μL; (%)	K/M: 0,16 – 0,8 (4,0 -8,0)
Liczba płytek krwi, x 10^3/μL	K/M: 150 – 350
Badania biochemiczne w surowicy	
IL-6, pg/mL	K/M: < 7,0**
Amylaza w surowicy, U/L	K/M: 62,0 - 220
Albumina, g/L	K/M: 35,0 – 50,0
Wapń całkowity, mmol/L	K/M: 2,02 – 2,61
Białko całkowite, g/L	K/M: 60,0 – 80,0
Bilirubina całkowita, μmol/L	K/M: 0 – 21,0
Mocznik, mmol/L	K/M: 2,76 – 8,07
Kreatynina, μmol/L	K/M: 45,0 – 97,0
sFlt-1, pg/mL	K/M: 63,0 – 108,0*
Angiopoetyna-2, ng/mL	K/M: 1,17 – 2,47
Prokalcytonina, ng/mL	K/M: < 0,1
Białko C-reaktywne, mg/L	K/M: < 5,0
LDH, U/L	K/M: 240,0 – 480,0
AST, U/L	K: 5,0 – 32,0/M: 5,0 – 40,0
ALT, U/L	K: 5,0 – 33,0/M: 5,0 – 41,0
Glukoza, mmol/L	K/M: 3,3 -5,6

uPAR, ng/mL	K/M: 1,195 – 4,415**
Badania biochemiczne w moczu	
KIM-1, ng/mL	K/M: 0,156 – 5,33**
Badanie układu krzepnięcia	
D-dimery, μg/mL	K/M: <0,5

*przedział wartości uzyskany w grupie 21 zdrowych ochotników; K- kobiety; M-mężczyźni;
** - zakres wartości podany przez producenta zestawu odczynnikowego

Streszczenie

Ostre zapalenie trzustki (OZT) jest jedną z najczęstszych ostrych chorób przewodu pokarmowego, która charakteryzuje się zróżnicowanym przebiegiem klinicznym – od postaci łagodnej, ustępującej samoistnie do ciężkiej, obarczoną śmiertelnością od 20-30%. Najnowsze doniesienia naukowe pokazują, że blisko połowa zgonów ma miejsce w ciągu pierwszego tygodnia od rozpoznania OZT i dotyczy głównie tych chorych, których od początku cechuje ciężki przebieg kliniczny schorzenia i u których dochodzi do rozwoju uogólnionej reakcji zapalnej (SIRS) powikłanej przez rozwijający się zespół niewydolności wielonarządowej (MODS). Spostrzeżenie to znalazło swoje odniesienie w zrewidowanej w 2012 roku klasyfikacji Atlanta wyróżniającej wczesną i późną fazę OZT. Faza wczesna obejmuje pierwszy tydzień, po którym rozwija się faza późna mogąca trwać tygodnie, a nawet miesiące. W ciągu pierwszych 7 dni za kluczowe dla dalszego przebiegu choroby uznaje się pierwsze 48 godziny trwania OZT. Podjęcie w tym czasie właściwego leczenia oraz rozpoznanie chorych, u których istnieje niebezpieczeństwo rozwoju ciężkiej postaci OZT (*severe acute pancreatitis – SAP*) daje szanse na obniżenie ryzyka ciężkich powikłań lub nawet zgonu. Działania diagnostyczne pomocne w prognozowaniu ciężkości przebiegu OZT powinny zatem obejmować pierwsze 48 godzin od rozpoczęcia choroby, a do przewidywania ciężkości przebiegu OZT w tym czasie wykorzystywane są zarówno badania obrazowe (tomografia komputerowa z kontrastem, rezonans magnetyczny, ultrasonografia), wieloczynnikowe skale prognostyczne (Ransona, APACHE II, Imrie-Glasgow, BISAP), jak również pojedyncze badania laboratoryjne (białko C-reaktywne, prokalcytonina, hematokryt). Ciągle jednak brak idealnego narzędzia do prognozowania przebiegu OZT.

Celem niniejszej pracy była ocena, w jakim stopniu oznaczenia interleukiny 6 (IL-6) oraz osoczowego receptora aktywatora plazminogenu typu urokinazowego (uPAR) mogą mieć znaczenie prognostyczne dla przewidywania ciężkiego przebiegu OZT u pacjentów we wczesnej fazie trwania OZT.

Do badania włączono łącznie 95 dorosłych chorych z OZT leczonych w Oddziale Chirurgicznym Szpitala Powiatowego w Wadowicach, u których czas trwania objawów do przyjęcia na oddział nie przekraczał 24 godzin. Materiał do badania pobierano w dniu przyjęcia oraz po 48 i 72 godzinach od wystąpienia objawów OZT. W oparciu o zmodyfikowaną klasyfikację Atlanta 2012, u 29 (30,5%) chorych zdiagnozowano łagodną postać OZT (*mild acute pancreatitis - MAP*), w przypadku 58 (61%) chorych postać średnio-ciężką (*moderately severe acute pancreatitis – MSAP*) oraz u 8 (8,5%) postać ciężką ostrego

zapalenia trzustki (*severe acute pancreatitis – SAP*). W trakcie leczenia 7 (7%) pacjentów wymagało przeniesienia na oddział intensywnego nadzoru. Łącznie w przebiegu badania zmarło 4 (4%) chorych, w tym 1 pacjent we wczesnej fazie, natomiast 3 chorych w późnej fazie trwania choroby.

W toku badania zebrano dane kliniczne oraz wyniki badań laboratoryjnych i obrazowych. Rutynowe badania laboratoryjne przeprowadzono w Laboratorium Zespołu Zakładów Opieki Zdrowotnej w Wadowicach, natomiast badania dodatkowe w Zakładzie Diagnostyki Szpitala Uniwersyteckiego oraz w Zakładzie Diagnostyki Katedry Biochemii Klinicznej UJ CM w Krakowie. Uzyskane wyniki badań pozwoliły na przygotowanie 3 prac oryginalnych opublikowanych w czasopismach naukowych indeksowanych w bazie PubMed oraz znajdujących się na liście Journal Citation Reports (Thomas Reuters) o łącznej wartości *Impact Factor* 9,270.

Pomiar stężenia IL-6 w surowicy oceniono w chwili przyjęcia oraz w kolejnym dniu hospitalizacji. U pacjentów z SAP w chwili przyjęcia obserwowano znamiennie wyższe stężenia IL-6 w surowicy w porównaniu do postaci MAP i MSAP. Dodatkowo, w obu dniach badania, stężenie IL-6 było dodatnio skorelowane z długością hospitalizacji, punktacją w skali Ransona, a także z wczesnymi markerami ostrej niewydolności nerek (AKI) tj. KIM-1 i L-FABP oraz markerami dysfunkcji śródbłonka (Ang-2 oraz sFlt-1). Potwierdzono również obecność korelacji pomiędzy stężeniem IL-6 w surowicy oraz dobrze rozpoznawanymi w praktyce klinicznej markerami stanu zapalnego tj. całkowitą liczbą leukocytów oraz bezwzględną liczbą neutrofili, stężeniem białka C-reaktywnego oraz prokalcytoniny. Ważnym aspektem przeprowadzonych badań była zaproponowana metodyka oznaczeń IL-6 w surowicy z wykorzystaniem w pełni zautomatyzowanej platformy analitycznej umożliwiającej monitorowanie zmian stężenia IL-6 w trybie diagnostyki rutynowej. Analiza dynamiki zmian stężenia IL-6 w surowicy prowadzona u chorych we wczesnej fazie rozwoju OZT wskazuje na jej znaczącą rolę jako czynnika prognostycznego wystąpienia ciężkiej postaci OZT, przetrwałej dysfunkcji narządowej (Marshall score ≥2), konieczności wdrożenia intensywnej terapii i śmiertelności w tej grupie chorych. Zastosowanie w pełni automatycznych metod oznaczeń stężenia IL-6 w surowicy umożliwia szybkie i powtarzalne oznaczanie stężenia IL-6 pozwalające na wykorzystanie wyników w diagnostyce różnicowej (*Int. J. Mol. Sci. 2018; 19: 1821; doi. 10.3390/ijms19061820).*

Celem *artykułu nr 2* było porównanie wartości diagnostycznej pojedynczych oznaczeń uPAR w surowicy z innymi wskaźnikami stanu zapalnego, jako czynników prognostycznych ciężkiego przebiegu OZT. Wykazano, że stężenie uPAR w surowicy w chwili przyjęcia

pozwala na prognozowanie SAP, niewydolności narządowej (głównie ostrego uszkodzenia nerek oraz niewydolności sercowo-naczyniowej), czy podjęcia decyzji o przeniesieniu pacjenta do oddziału intensywnej opieki medycznej. Wykazano również istnienie korelacji pomiędzy stężeniem uPAR w surowicy oraz laboratoryjnymi markerami uszkodzenia wątroby tj. AST, ALT i bilirubiną. Analiza użyteczności diagnostycznej testu z oceną wielkości pola pod krzywą ROC (AUC) dla uPAR wykazała brak znaczących różnic w porównaniu do innych badanych markerów tj. IL-6, CRP, PCT, D-dimerów oraz sFlt-1. Do oznaczeń uPAR w surowicy wykorzystywano technikę pomiaru ELISA. Trzeba podkreślić, że obecnie brak jest standaryzacji laboratoryjnej metod oznaczania uPAR. Pomimo obiecujących wstępnych wyników, należy ostrożnie formułować wnioski końcowe w zakresie wdrożenia oznaczeń uPAR w celu prognozowaniu SAP. Badania wymagają potwierdzenia dotychczasowych obserwacji w grupie o większej liczebności, w szczególności pacjentów z SAP. Na tę chwilę należy uznać, że oznaczanie stężenia uPAR w surowicy w celu prognozowania ciężkości przebiegu OZT we wczesnej fazie rozwoju choroby nie wnosi większych korzyści od stosowanych rutynowo i uznanych testów tj. CRP, PCT, czy D-dimery (*J. Clin. Med. 2018; 7: 305; doi. 10.3390/jcm7100305*).

W *artykule nr 3* przeprowadzono ocenę korelacji pomiędzy stężeniami IL-6 i uPAR w surowicy oraz wskaźnikami wyliczanymi w oparciu o bezwzględne liczby neutrofili do limfocytów (NLR), limfocytów do monocytów (LMR) oraz płytek krwi do limfocytów (PLR) u chorych z OZT we wczesnej fazie trwania choroby. Wskaźniki wyliczano w oparciu o wykonywane rutynowo badania morfologii krwi z rozmazem w każdym z 3 dnia badania. W oparciu o zmodyfikowaną skalę Marshalla (MMSS), u pacjentów z niewydolnością narządową (MMSS≥2 punktów) wykazano znamiennie statystycznie niższą wartość wskaźnika LMR w pierwszej dobie trwania OZT. W drugim i trzecim dniu badania obserwowano znamiennie wyższe wartości wskaźnika NLR u pacjentów o cięższym przebiegu OZT. Analiza korelacji wykazała istnienie dodatniej korelacji pomiędzy wartością wskaźnika NLR oraz stężeniami IL-6, PCT oraz uPAR w każdym dniu badania. W każdym dniu badania obserwowano również ujemną korelację pomiędzy bezwzględną liczbą limfocytów oraz stężeniami IL-6, PCT oraz uPAR w surowicy krwi chorych. Dodatkowo, po upływie 48 godzin obserwacji potwierdzono obecność korelacji pomiędzy całkowitą liczbą leukocytów, bezwzględną liczbą neutrofili oraz wskaźnikiem NLR ze stopniem ciężkości chorych ocenianym wg. skali Ransona (*Folia Med.Cracov. 2018; 4: 57-74; doi. 10.24425/fmc.2018125704*).

Summary

Acute pancreatitis (AP) is one of the most common acute digestive tract diseases with diverse clinical outcomes – from mild resolving on its own to severe characterized by 20-30% mortality rate. Recent reports suggest that nearly half of deaths occur in the first week from AP diagnosis and concern mainly patients who from the beginning present with severe course of the disease and develop systemic inflammatory response syndrome (SIRS) complicated by multiple organ dysfunction syndrome (MODS). This observation has been reflected in the revised 2012 Atlanta classification, which differentiates between the early and the late phase of AP. The early phase includes the first week, followed by the late phase lasting weeks or even months. The first 48 hours of the early phase are crucial for further disease progression. Adequate treatment and identification of patients at risk of developing the severe form of AP (*severe acute pancreatitis*, SAP) within this time frame decreases the risk of serious complications or even death. Therefore, the diagnostic evaluation of AP severity should occur within the first 48 hours from the onset of symptoms. At present, it involves clinical assessment, imaging tests (contrast-enhanced computed tomography, magnetic resonance imaging, ultrasonography), multi-variable predictive scores (Ranson, APACHE II, Imrie-Glasgow, BISAP) and single laboratory markers (C-reactive protein, procalcitonin, hematocrit). All the above diagnostic tools, however, have significant limitations.

The aim of the study was to evaluate the diagnostic utility of interleukin-6 (IL-6) and serum urokinase-type plasminogen activator receptor (uPAR) measurements for the prediction of the severe course of AP in patients in the early phase of AP.

The study included 95 adult patients with AP treated at the Surgery Unit, Complex of Health Care Centers in Wadowice, Poland whose symptoms lasted less than 24 hours preceding admission. Blood samples were collected on admission and after 48 and 72 hours from the onset of AP symptoms. Based on the revised Atlanta 2012 classification, 29 (30.5%) patients were diagnosed with mild acute pancreatitis (MAP), 58 (61%) with moderately severe acute pancreatitis (MSAP) and 8 (8.5%) with severe acute pancreatitis (SAP). During treatment 7 (7%) patients required transfer to an intensive care unit (ICU). Throughout the study 4 (4%) patients died in the early phase and 3 in the late phase of the disease.

In the course of the study, clinical data, laboratory and imaging test results were collected. The routine laboratory tests were conducted in the Diagnostic Laboratory of Complex of Health Care Centers in Wadowice, whereas additional tests were performed at the Diagnostics Department of University Hospital in Krakow and the Department of Diagnostics

of Chair of Clinical Biochemistry of the Jagiellonian University Medical College in Krakow. Based on the obtained results, three original articles were published in scientific journals indexed in PubMed and included in the Journal Citation Reports (Thomas Reuters) with the total Impact Factor of 9.270.

IL-6 concentrations in sera were evaluated on admission and on the following day of hospitalization. In patients with SAP, significantly higher IL-6 concentrations were observed on admission when compared with MAP and MSAP group. Moreover, on both days of the study, concentrations of IL-6 correlated with the duration of the hospital stay, Ranson's score, KIM-1 and L-FABP – early markers of acute kidney injury (AKI) – as well as markers of endothelial dysfunction (Ang-2 and sFlt-1). There was a correlation between the IL-6 concentrations in the serum and inflammatory markers widely recognized in the clinical practice such as total leukocytes count, absolute neutrophil count, C-reactive protein and procalcitonin concentrations. A significant element of the study was the methodology proposed for IL-6 measurements, i.e. the use of fully automated analytical platform which allows for IL-6 monitoring in routine laboratory diagnostics. The analysis of the dynamics of changes in serum concentrations of IL-6 conducted in patients in the early phase of AP indicates its significant role as a prognostic factor of the disease progression into the severe form of AP, persistent organ failure (Marshall score ≥2) and the need for intensive care and mortality. The use of fully automated IL-6 assay allows for fast and repeatable results which could be incorporated in routine diagnostic reasoning (*Int. J. Mol. Sci. 2018; 19: 1821; doi. 10.3390/ijms19061820*).

The aim of *article 2* was to compare the diagnostic usefulness of single measurements of serum uPAR with other inflammatory markers in the prognosis of severe course of AP. It was shown that the uPAR concentrations on admission allow for the prognosis of SAP, organ failure (primarily acute kidney injury and acute cardiovascular failure) or the decision to transfer the patient to an intensive care unit. Correlations were shown between the serum uPAR and laboratory markers of liver injury such as AST, ALT and bilirubin. The analysis of the diagnostic utility with the evaluation of the area under the ROC curve (AUC) for uPAR did not show significant differences from other studied biomarkers: IL-6, CRP, PCT, D-dimers and sFlt-1. The serum uPAR measurements were conducted using the ELISA and until now, uPAR measurements by various methods are not standardized. Despite promising preliminary results, there is no possibility to draw definitive conclusions as for wider uPAR application in SAP prediction. Further studies on a larger group of patients (especially those with SAP) are needed. Meanwhile, however, it must be said that serum uPAR measured in the

early phase of the disease does not outperform the routinely conducted and widely acknowledged laboratory tests such as CRP, PCT, or D-dimers in the prediction of the severe course of AP (*J. Clin. Med. 2018; 7: 305; doi. 10.3390/jcm 7100305*).

In *article 3*, correlation analysis was conducted between the serum concentrations of IL-6 and uPAR and the ratios of absolute neutrophil-to-lymphocyte count (NLR), lymphocyte-to-monocyte count (LMR) and platelet-to-lymphocyte count (PLR) in AP patients in the early phase of the disease. The indexes were calculated based on the routinely conducted complete blood counts on each of the 3 study days. Based on the modified Marshall score (MMSS), in patients with organ failure (MMSS ≥2) LMR was significantly lower in the first 24 hours of AP. On the day 2 and 3, significantly higher values of NLR were observed in patients with severe course of the disease. There was a positive correlation between NLR value and IL-6, PCT and uPAR concentrations throughout the study. On each of 3 days, negative correlations were also observed between the absolute lymphocyte count and serum IL-6, PCT and uPAR concentrations. Additionally, after 48 hours of observation, correlations were confirmed between the total leukocyte counts, absolute neutrophil counts, NLR and the AP severity evaluated according to Ranson's score (*Folia Med. Cracov. 2018; 4: 57-74; doi. 10.24425/fmc.2018125704*).