NARODOWY INSTYTUT ONKOLOGII IM. MARII SKŁODOWSKIEJ-CURIE - PAŃSTWOWY INSTYTUT BADAWCZY W WARSZAWIE

CIESZYMIERZ GAWIŃSKI

„Rola markerów ogólnoustrojowej reakcji zapalnej LMR (lymphocyte-to-monocyte ratio), NLR (neutrophil-to-lymphocyte ratio) i PLR (platelet-to-lymphocyte ratio) w morfologii krwi obwodowej u pacjentów z rakiem jelita grubego i odbytnicy.”

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Serdecznie dziękuję prof. dr. hab. n. med. Lucjanowi Wyrwiczowi oraz wszystkim współautorom za pomoc przy pisaniu prac i wsparcie merytoryczne.

Niniejszą pracę chciałbym zadedykować Rodzicom i Żonie.

SPIS TREŚCI

Wykaz skrótów i akronimów użytych w pracy oraz ich objaśnienia

AJCC – Amerykański Wspólny Komitet ds. Raka (ang. *American Joint Committee on Cancer*)

CEA- antygen karcynoembrionalny (ang. *carcinoembryonic antigen*)

CPS – stosunek komórek z ekspresją PD-L1 (komórek nowotworowych, limfocytów, makrofagów) do wszystkich komórek nowotworowych pomnożony przez 100 (*ang. combined positive score*)

CRC – rak jelita grubego i odbytnicy (ang. *colorectal cancer*)

CRI – reakcja zapalna związana z nowotworem (ang. *cancer-related inflammation*)

DFS - czas przeżycia wolny od choroby (ang. *disease-free survival*)

EMVI – naciekanie naczyń zlokalizowanych poza ścianą odbytnicy (ang. *extramural vascular invasion*)

LARC – miejscowo zaawansowany rak odbytnicy (ang. *locally-advanced rectal cancer*)

LMR – stosunek limfocytów do monocytów (ang. *lymphocyte-to-monocyte ratio*)

MRI – badanie rezonansu magnetycznego (ang. *magnetic resonance imaging*)

NLR – stosunek neutrocytów do limfocytów (ang. *neutrophil-to-lymphocyte ratio*)NK

OS – czas przeżycia całkowitego (ang. *overall survival*)

pCR – patologiczna całkowita odpowiedź na leczenie (ang. *pathological complete response*)

PD-L1 – ligand programowanej śmierci 1 (*ang. programmed death-ligand 1*)

PLR – stosunek trombocytów do limfocytów (ang. *platelet-to-lymphocyte ratio*)

RFS – czas do wznowy choroby nowotworowej (ang. *reccurence-free survival*)

SIR – ogólnoustrojowa reakcja zapalna (ang. *systemic inflammatory response*)

TIICs - Komórki układu immunologicznego naciekające guz (ang. *tumor-infiltrating immune cells*)

TILs - Limfocyty naciekające guz (ang. *tumor-infiltrating lymphocytes*)

TME – operacja całkowitego usunięcia mezorektrum (ang. *total mesorectal excision*)

TNM – system klasyfikacji zaawansowania klinicznego nowotworu (ang. *tumor, node, metastasis*)

UICC – Międzynarodowa Unia do Walki z Rakiem (ang. *Union for International Cancer Control*)

Streszczenie

Cel Pracy

Celem prowadzonych badań było określenie roli wykładników ogólnoustrojowej reakcji zapalnej (systemic inflammatory response, SIR): stosunku limfocytów do monocytów (lymphocyte-to-monocyte ratio, LMR), stosunku neutrocytów do limfocytów (neutrophil-to-lymphocyte ratio, NLR) i stosunku trombocytów do limfocytów (platelet-to-lymphocyte ratio, PLR) u pacjentów z rakiem jelita grubego i odbytnicy (colorectal cancer, CRC). Ocenie poddawano powtarzalność, wartość prognostyczną, relacje z innymi parametrami biochemicznymi, radiologicznymi, klinicznymi i patologicznymi. Analiza zbioru prac służyła określeniu możliwości zastosowania wykładników SIR w praktyce lekarskiej.

Materiały i metody

We wszystkich badaniach wchodzących w skład niniejszej rozprawy doktorskiej brali udział pacjenci leczeni w Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie − Państwowym Instytucie Badawczym w Warszawie.

W 1. publikacji prospektywnej analizie poddano 60 wyselekcjonowanych pacjentów z miejscowo zaawansowanym rakiem odbytnicy leczonych między sierpniem 2017r. a grudniem 2020r. Celem analizy była ocena powtarzalności LMR, NLR i PLR i ich wartości prognostycznej, korelacji z parametrami biochemicznymi, klinicznymi oraz patologicznymi.

W 2. publikacji 371 pacjentów z miejscowo zaawansowanym rakiem odbytnicy leczonych między sierpniem 2016r. a grudniem 2021r. oceniono retrospektywnie pod kątem związków między wykładnikami SIR, parametrami klinicznymi i laboratoryjnymi a radiologicznym czynnikiem prognostycznym - naciekaniem naczyń zlokalizowanych poza ścianą odbytnicy (extramural vascular invasion, EMVI).

W 3. publikacji retrospektywnej analizie poddano 87 pacjentów z lewostronnymi miejscowo zaawansowanym rakiem jelita grubego lub górnej odbytnicy leczonych operacyjnie między styczniem 2014r. a grudniem 2015r. Celem wykluczenia wpływu radioterapii na wykładniki SIR, przeprowadzono badanie na populacji pacjentów poddawanych pierwotnemu leczeniu chirurgicznemu. Oceniano korelacje między parametrami LMR, NLR i PLR a gęstością limfocytów CD3+ i CD8+ w mikrośrodowisku guza.

Wyniki

W 1. publikacji wykazano znamienną statystycznie zależność pomiędzy LMR a naciekiem zapalnym w obrębie guza ($r = 0,38$, $p = 0,044$) i ekspresją liganda programowanej śmierci 1 (programmed death-ligand 1, PD-L1) na komórkach nowotworowych, limfocytach i makrofagach przy użyciu wskaźnika CPS (combined positive score) ($r = 0,45$, $p = 0,016$). Wartość PLR była istotnie związana z zajęciem przez nowotwór regionalnych węzłów chłonnych ($p = 0,033$). Wykładniki SIR okazały się być umiarkowanie powtarzalne. Nie wykazano wartości prognostycznych w stosunku do czasu przeżycia całkowitego (overall survival, OS) oraz czasu do wznowy choroby nowotworowej (reccurence-free survival, RFS).

W 2. publikacji stwierdzono znamienną statystycznie zależność między wielkością guza nowotworowego, przerzutami do regionalnych węzłów chłonnych, stopniem zaawansowania choroby nowotworowej a obecnością EMVI ($p < 0,001$). Wyjściowy poziom neutrocytów, trombocytów i antygenu karcynoembrionalnego (carcinoembryonic antigen, CEA) był istotnie wyższy w populacji pacjentów EMVI-dodatnich w stosunku do populacji pacjentów EMVI-ujemnych (odpowiednio $p = 0,041$, $p = 0,01$, $p = 0,027$). Nie zaobserwowano istotnych różnic w wartościach LMR, NLR i PLR między pacjentami EMVI-dodatnimi i EMVI-ujemnymi.

W publikacji 3. udowodniono znamienną statystycznie zależność między gęstością limfocytów CD3+ w centrum guza a wyjściową wartością NLR ($p = 0,044$). Nie zaobserwowano innych zależności między wartościami LMR, NLR lub PLR a gęstością limfocytów CD3+ i CD8+. Wśród pacjentów z wysokim LMR, niskim NLR i niskim PLR odsetek pięcioletnich OS był znamiennie wyższy (odpowiednio $p < 0,001$, $p = 0,001$, $p=0,095$). Nie stwierdzono zależności między OS a gęstością limfocytów CD3+ i CD8+ w mikrośrodowisku guza.

Wnioski

Wykładniki SIR są parametrami o nie w pełni poznanych właściwościach. Dane literaturowe na temat zagadnień poruszanych w pracach są bardzo skąpe. Na podstawie prezentowanych publikacji można przypuszczać, że istnieje zależność między wykładnikami SIR w morfologii krwi obwodowej a zaburzeniami immunologicznymi związanymi z chorobą nowotworową w mikrośrodowisku guza. LMR, NLR i PLR mają potwierdzone właściwości prognostyczne w lewostronnym raku jelita grubego, a poziom ich powtarzalności umożliwia zastosowanie w codziennej praktyce klinicznej. Zbiór zaprezentowanych prac powinien być wstępem do dalszych badań nad rolą wykładników SIR w CRC.

Abstract

Objective

The aim of the studies was to assess the role of systemic inflammatory response (SIR) markers: lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) in patients with colorectal cancer (CRC). Their reproducibility, prognostic value, correlations with biochemical, radiological, clinical and pathological parameters were evaluated. The purpose of the analysis of the studies was to assess the utility of SIR markers in clinical practice.

Materials and methods

Patients treated at the Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw were enrolled in the studies.

In study 1. sixty well-selected patients with locally-advanced rectal cancer treated between August 2017 and December 2020 were prospectively analyzed. The aim of the analysis was the evaluation of the reproducibility of the LMR, NLR and PLR, their prognostic values and correlations with clinical, biochemical and pathological outcomes.

In study 2. three hundred seventy-one patients with locally-advanced rectal cancer treated between August 2016 and December 2021 were retrospectively assessed in terms of correlations between SIR markers, clinical and laboratory parameters and radiological prognostic factor – extramural vascular invasion (EMVI).

In the 3. study 87 patients with left-sided colon cancers or upper rectal cancers treated surgically between January 2014 and December 2015 were analyzed. The aim of the study was to exclude the impact of radiotherapy on SIR markers by assessing a population of patients treated primarily with surgery. The correlations between the LMR, NLR and PLR and the density of CD3+ and CD8+ lymphocytes in the tumor microenvironment were assessed.

Results

In the 1. study there was a significant positive correlation between the LMR and cancer-related inflammatory infiltrate ($r = 0.38$, $p = 0.044$) and programmed death-ligand 1 (PD-L1) expression in tumor cells, lymphocytes, and macrophages, assessed as CPS (combined positive score) ($r = 0.45$, $p =$

0.016). The PLR level was correlated with nodal involvement ($p = 0.033$). The SIR markers proved to be moderately reproducible; there was no prognostic value in terms of overall survival (OS) and recurrence-free survival (RFS).

In the 2. study a correlation between the extension of the tumor, nodal status, clinical stage of the disease and the presence of EMVI was found ($p < 0.001$). The pre-treatment level of neutrophils, platelets and carcinoembryonic antigen (CEA) was significantly higher in the EMVI-positive population compared to EMVI-negative population ($p = 0.041$, $p = 0.01$, $p = 0.027$, respectively). There were no significant differences regarding the level of the LMR, NLR and PLR between the EMVI-positive and EMVI-negative population.

In the 3. study a statistically significant correlation between the density of CD3+ lymphocytes in the center of the tumor and the pre-treatment level of the NLR was proven ($p = 0.044$). No other associations between any of the SIR markers and CD3+ or CD8+ lymphocytes were observed. Five-year OS was significantly longer in patients with the high LMR ($p < 0.001$), low NLR ($p = 0.001$) and low PLR ($p = 0.095$). No correlations between the density of lymphocytes CD3+ and CD8+ in the tumor microenvironment and OS was demonstrated.

Conclusions

The properties of the SIR markers are not entirely discovered. In the literature there is little data on the topics presented in the studies. Based on our results, we may assume there is an association between the SIR markers in the blood and cancer-related immune disorders in the tumor microenvironment. LMR, NLR and PLR have a confirmed prognostic value in left-sided colon cancer, their reproducibility enables application in everyday clinical practice. The presented works should serve as an introduction to further studies of the role of the SIR markers in CRC.

Wykaz publikacji wchodzących w skład rozprawy doktorskiej

Niniejsza rozprawa doktorska opiera się na zbiorze publikacji dotyczących wykładników ogólnoustrojowej reakcji zapalnej u pacjentów z rakiem jelita grubego i odbytnicy. W skład zbioru publikacji wchodzą trzy prace oryginalne (1 prospektywna i 2 retrospektywne) opublikowane w zagranicznych, recenzowanych czasopismach naukowych:

1. Gawiński, C.; Mróz, A.; Roszkowska-Purska, K.; Sosnowska, I.; Derezińska-Wołek, E.; Michalski, W.; Wyrwicz, L. A Prospective Study on the Roles of the Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), and Platelet-to-Lymphocyte Ratio (PLR) in Patients with Locally Advanced Rectal Cancer. *Biomedicines* **2023**, *11*, 3048. https://doi.org/10.3390/biomedicines11113048
   (***IF – 4.7***)

2. Gawiński, C.; Hołdakowska, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Extramural Vascular Invasion (EMVI) in Locally Advanced Rectal Cancer. *Curr. Oncol.* **2023**, *30*, 545-558. https://doi.org/10.3390/curroncol30010043
   (***IF – 2.6***)

3. Gawiński, C.; Michalski, W.; Mróz, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Tumor-Infiltrating Lymphocytes (TILs) in Left-Sided Colorectal Cancer Patients. *Biology* **2022**, *11*, 385. https://doi.org/10.3390/biology11030385
   (***IF– 5.2***)

   Łączny IF publikacji – 12.5

Wstęp i założenia pracy

Rak jelita grubego i odbytnicy (colorectal cancer, CRC) jest trzecim najpowszechniej występującym nowotworem i drugą najczęstszą nowotworową przyczyną zgonów na świecie (1). Rak odbytnicy stanowi około 35% przypadków CRC (2). W ostatnich latach obserwuje się niepokojący wzrost zachorowań na CRC wśród młodych dorosłych (3, 4). Rokowanie, szczególnie w zaawansowanych stadiach choroby pozostaje niesatysfakcjonujące (5). Standardem postępowania w miejscowo zaawansowanym raku jelita grubego jest pierwotne leczenie operacyjne, zaś w przypadku miejscowo zaawansowanego raka odbytnicy (locally-advanced rectal cancer, LARC) neoadjuwantowa radio- lub radiochemioterapia, następnie operacja polegająca na całkowitym usunięciu mezorektum (total mesorectal excision, TME) z lub bez pooperacyjnej chemioterapii uzupełniającej (6-8). Wpływ takiego postępowania w leczeniu LARC na czas przeżycia całkowitego (overall survival, OS) pozostaje niejasny, a intensywne, multimodalne leczenie może powodować istotne, długotrwałe powikłania i w konsekwencji znaczne pogorszenie jakości życia (9, 10). Wątpliwości te powodują występowanie znaczących różnic w postępowaniu terapeutycznym z pacjentami z LARC między ośrodkami medycznymi. Klasyfikacja TNM (tumor, node, metastasis) zaawansowania klinicznego wg UICC (*Union for International Cancer Control*) i AJCC (American Joint Committee on Cancer) jest podstawowym czynnikiem prognostycznym i parametrem służącym do oceny zaawansowania choroby i planowania leczenia (11). Klasyfikacja TNM często nie wystarcza, by właściwie ocenić faktyczne zaawansowanie choroby. Zachodzi konieczność znalezienia nowych markerów, które pomogłyby lepiej dostosować intensywność leczenia do potrzeb danego pacjenta. Markery reakcji zapalnej związanej z nowotworem (cancer-related inflammation, CRI) zarówno lokalnie w mikrośrodowisku guza, jak i obwodowo w krwioobiegu, mogą być odpowiednimi kandydatami do tej roli. Stosunek limfocytów do monocytów (lymphocyte-to-monocyte ratio, LMR), neutrocytów do limfocytów (neutrophil-to-lymphocyte ratio, NLR), trombocytów do limfocytów (platelet-to-lymphocyte ratio, PLR) to parametry oceniane na podstawie morfologii krwi obwodowej z udowodnionymi właściwościami prognostycznymi w wielu nowotworach (12-14).

CRI jest kluczowym zjawiskiem dla zrozumienia właściwości markerów ogólnoustrojowej reakcji zapalnej (systemic inflammatory response, SIR). Związek między chorobą nowotworową i reakcją zapalną jest znany od czasu badań Rudolfa Virchowa w XIX wieku (15). Rekrutacja komórek zapalnych, produkcja cytokin, reaktywnych form tlenu i hamowanie programów naprawy komórkowej promują niekontrolowaną proliferację wadliwych komórek, a przez to potencjał do nowotworzenia. Komórki zapalne są powszechne w mikrośrodowisku guza (16). Liczba limfocytów odzwierciedla nasilenie reakcji zapalnej. Limfocyty odpowiadają za produkcję cytokin przeciwnowotworowych, a ich aktywność cytotoksyczna zmniejsza proliferację nowotworu i tendencję do rozsiewu (17). Przeciwnie, monocyty przyczyniają się do progresji nowotworu i zwiększają jego aktywność metastatyczną (18).

Neutrocyty, które stanowią ok. 50-70% leukocytów, odgrywają kluczową rolę w CRI. Wydzielając reaktywne formy tlenu i azotu uszkadzające DNA biorą one istotny udział w procesie nowotworzenia. Rozwój nowotworu jest przyspieszany przez wydzielane przez neutrocyty cytokiny wpływające na proces angiogenezy (19). Neutrocyty biorą także udział w hamowaniu proliferacji limfocytów T i limfocytów NK (20, 21). Podobnie trombocyty, poprzez wydzielanie cytokin i czynników wzrostu, przyczyniają się do karcynogenezy. Komórki nowotworowe produkują cytokiny, które indukują trombocytozę. Trombocyty z kolei promują, dzięki wspomnianym mechanizmom, dalszy wzrost guza przyczyniając się do błędnego koła stymulacji (22). Opisane interakcje immunologiczne doprowadziły do zainteresowania i rozpoczęcia badań nad wspomnianymi markerami SIR – LMR, NLR i PLR. Pomimo udowodnionej roli prognostycznej markerów SIR w wielu nowotworach, ich związki z innymi parametrami kliniczno-patologicznymi, radiologicznymi lub biochemicznymi oraz możliwość zastosowania w praktyce klinicznej pozostają niejasne.

Komórki układu immunologicznego naciekające guz (tumor-infiltrating immune cells, TIICs) składają się głównie z makrofagów, komórek dendrytycznych, mastocytów i limfocytów. Limfocyty naciekające guz (tumor-infiltrating lymphocytes, TILs) to białe krwinki migrujące z krwioobiegu w stronę guza nowotworowego, które są obecnie szeroko badaną populacją TIICs. TILs są zaangażowane w rozpoznawanie i eliminację komórek nowotworowych odgrywając ważną rolę w nasilaniu odpowiedzi immunologicznej skierowanej przeciwko nowotworowi (23). Ich prognostyczna i predykcyjna rola jest dobrze udokumentowana w raku piersi, szczególnie w podtypie trójujemnym, gdzie wysoki poziom TILs koreluje z dłuższym OS, dłuższym czasem przeżycia wolnego od choroby (disease-free survival, DFS) oraz częstszym występowaniem patologicznej całkowitej odpowiedzi po leczeniu neoadjuwantowym (24, 25). Wysoki poziom TILs jest również związany z lepszym rokowaniem w innych nowotworach takich jak rak płuca, jajnika czy trzustki (26-28). Immunoscore to klasyfikacja oparta na populacjach limfocytów w mikrośrodowisku guza (CD3+, CD8+, CD45RO+) ocenianych immunohistochemicznie w obrębie guza (29). Według niektórych doniesień Immunoscore jest lepszym predyktorem OS w porównaniu z klasyfikacją TNM w CRC (30). W literaturze dane dotyczące związków między obwodowymi markerami SIR, a zmianami związanymi z CRI w mikrośrodowisku guza są sprzeczne. W raku piersi stwierdzono istotną korelację między poziomem limfocytów, monocytów, LMR, NLR we krwi obwodowej a poziomem TILs (CD3+, CD8+, CD15+, CD68+) (31, 32). Wysoki przedoperacyjny poziom NLR był związany z niskim poziomem TILs w raku wątrobowokomórkowym (33). Z kolei w raku żołądka nie stwierdzono korelacji między gęstością limfocytów CD3+ i CD8+ a poziomem NLR. W CRC dane na temat zależności między TILs a markerami SIR są bardzo skąpe. W pojedynczych badaniach wysokie wartości LMR były związane z wysokim poziomem limfocytów CD3+ (34). W raku odbytnicy nie stwierdzono zależności między poziomem NLR a obecnością CD3+ (35).

W raku odbytnicy istotnym negatywnym czynnikiem prognostycznym jest naciekanie naczyń zlokalizowanych poza ścianą odbytnicy (extramural vascular invasion, EMVI). Wykrycie EMVI w

przedoperacyjnym badaniu rezonansu magnetycznego (magnetic resonance imaging, MRI) jest związane ze zwiększonym ryzykiem wystąpienia przerzutów odległych i skróconym DFS (36). Obecność EMVI jest skorelowana z zaawansowanymi stadiami choroby (wielkość guza, zajęcie regionalnych węzłów chłonnych) (37-39). Tradycyjnie EMVI oceniane było na podstawie badania patomorfologicznego. Jednakże udowodniono, że taki sposób oceny prowadzi do znacznego zaniżania częstości występowania naciekania naczyń. Historyczne badania szacują częstość występowania EMVI w przedziale od 9% do 90%, co jest związane z nieustandaryzowaniem kryteriów patomorfologicznych oraz trudnościami w rozróżnieniu pomiędzy naciekiem naczyń limfatycznych i żylnych. W ostatnich latach wykazano, że identyfikowanie EMVI na podstawie MRI jest co najmniej tak samo dokładne jak w ocenie patomorfologicznej (40, 41). Ocena parametru EMVI stała się standardową procedurą w ramach przedoperacyjnego badania MRI w raku odbytnicy, a częstość występowania tego parametru w ocenie radiologicznej wynosi ok. 35% (42, 43). Ocena EMVI jest coraz częściej używana jako czynnik wskazujący pacjentów wymagających bardziej intensywnego leczenia okołooperacyjnego. Chand i wsp. wykazali, że pacjenci z EMVI-dodatnim rakiem odbytnicy w II stopniu zaawansowania wg TNM mają podobnie podwyższone ryzyko wystąpienia przerzutów odległych jak pacjenci z EMVI-negatywnym rakiem w st. III choroby (44). Siddiqui i wsp. w metaanalizie wykazali istotnie zwiększone ryzyko wystąpienia zarówno synchronicznych jak i metachronicznych przerzutów wśród pacjentów z EMVI-dodatnim rakiem odbytnicy (43). Rola EMVI nie jest ograniczona do raka odbytnicy - jest także negatywnym czynnikiem prognostycznym w miejscowo zaawansowanym raku jelita grubego. Badania wskazują też na potencjalną wartość prognostyczną EMVI w raku przełyku i żołądka (45-47). Dane w literaturze na temat relacji pomiędzy parametrem EMVI a markerami SIR są bardzo skąpe. W badaniu Li i wsp. nie wykazano związków między EMVI a poziomem NLR i PLR w LARC (48). W innym badaniu Pine i wsp. stwierdzili statystycznie znamienną zależność między NLR a EMVI. W analizie tej ujęto jednak parametr EMVI oceniany na podstawie badania patomorfologicznego a nie radiologicznego, zaś populacja pacjentów nie ograniczała się do chorych z rakiem odbytnicy (49). Ocena korelacji między markerami SIR a parametrem EMVI mogłaby ułatwić przedoperacyjną stratyfikację ryzyka u pacjentów z LARC.

Cel pracy

Markery SIR – LMR, NLR i PLR mają udowodnioną wartość prognostyczną w wielu nowotworach. W związku z tym, że parametry te oceniane są na podstawie morfologii krwi obwodowej, ich pozyskiwanie i analiza jest łatwa i tania. Ich rola w miejscowo zaawansowanym CRC, szczególnie LARC nie jest jednak dobrze określona. Jako potencjalne markery prognostyczne wymagają właściwej walidacji – przede wszystkim oceny powtarzalności w praktyce klinicznej. Głównym celem badań wchodzących w skład niniejszej rozprawy doktorskiej było zrozumienie roli markerów SIR wśród pacjentów z miejscowo zaawansowanym CRC. Ocenie poddawano powtarzalność markerów SIR w dobrze wyselekcjonowanej grupie pacjentów z rakiem odbytnicy. Analizowano związki między LMR, NLR i PLR a innymi parametrami kliniczno – patologicznymi, radiologicznymi i biochemicznymi. Poszukiwano korelacji między obwodowymi markerami CRI, a zaburzeniami immunologicznymi w mikrośrodowisku guza. Wyniki prac miały na celu ocenę możliwości zastosowania markerów SIR w codziennej praktyce lekarskiej.

Materiały i metody

We wszystkich badaniach wchodzących w skład niniejszej pracy doktorskiej udział brali pacjenci leczeni w Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie − Państwowym Instytucie Badawczym w Warszawie.

Publikacja 1. miała charakter jednoramiennego badania prospektywnego. Poddano ocenie 60 wyselekcjonowanych pacjentów z miejscowo zaawansowanym rakiem odbytnicy leczonych między sierpniem 2017r. a grudniem 2020r. Kryteriami włączenia do badania były: a) miejscowo zaawansowany rak odbytnicy; b) kwalifikacja pacjentów do otrzymania radio- lub radiochemioterapii przez zespół wielodyscyplinarny. Kryteria wyłączenia z badania stanowiły: a) obecność współwystępujących nowotworów; b) występowanie ostrego lub przewlekłego stanu zapalnego, chorób hematologicznych lub autoimmunologicznych i innych stanów medycznych mogących istotnie wpływać na zaburzenia wykładników stanu zapalnego; c) stosowanie leczenia immunosupresyjnego w wywiadzie. U wszystkich pacjentów przeprowadzano trzy pobrania morfologii krwi przed rozpoczęciem leczenia onkologicznego. Na podstawie wyników morfologii oceniano LMR, NLR i PLR. Wszyscy pacjenci otrzymali radio- lub radiochemioterapię, w zależności od stopnia zaawansowana choroby, zgodnie z decyzją zespołu wielodyscyplinarnego. Dziesięciu pacjentów nie zgodziło się na zabieg operacyjny. U 6 pacjentów doszło do progresji choroby nowotworowej lub choroba okazała się być nieoperacyjna po leczeniu neoadjuwantowym. Operacji poddanych zostało 44 pacjentów. Analizie poddawano pooperacyjny materiał histopatologiczny. W 10 przypadkach doszło do całkowitej odpowiedzi patologicznej (pathological complete response, pCR). W 5 przypadkach materiał

histopatologiczny był niedostępny lub uznany za nieodpowiedni do oceny patomorfologicznej. W pozostałych 29 preparatach histopatologicznych oceniono obecność limfocytów CD8+, nacieku zapalnego (limfocytów, plazmocytów, monocytów/makrofagów oraz neutrocytów), białek MSH6 i/lub PMS2 oraz wskaźnik CPS (combined positive score) - stosunek komórek (komórek nowotworowych, limfocytów i makrofagów) z ekspresją liganda programowanej śmierci komórki (programmed death-ligand 1, PD-L1) do wszystkich komórek nowotworowych pomnożony przez 100. Ocenie poddawano powtarzalność LMR, NLR i PLR, ich wartość prognostyczną oraz związek z czynnikami klinicznymi i wspomnianymi powyżej parametrami patomorfologicznymi.

Publikacja 2. miała charakter retrospektywny. Analizie poddano 371 pacjentów z LARC leczonych między sierpniem 2016r. a grudniem 2021r. Kryteriami włączenia do badania były: a) miejscowo zaawansowany rak odbytnicy; b) diagnostyka z zastosowaniem MRI wysokiej rozdzielczości z oceną statusu EMVI; c) kwalifikacja do leczenia radio- lub radiochemioterapią przez zespół wielodyscyplinarny. Kryteria wyłączenia obejmowały: a) obecność współwystępujących nowotworów; b) obecność zaburzeń hematologicznych lub innych stanów chorobowych mogących istotnie wpłynąć na wykładniki stanu zapalnego; c) stosowanie leczenia immunosupresyjnego w wywiadzie. Mediana czasu między badaniem morfologii krwi, na podstawie którego wyliczano LMR, NLR i PLR a MRI wynosiła 8 dni (zakres 0 - 43 dni). Ocena EMVI była przeprowadzana przez dwóch niezależnych radiologów z co najmniej 10-letnim doświadczeniem. Analizowano związki parametrem EMVI a LMR, NLR i PLR oraz markerami biochemicznymi i parametrami kliniczno-patologicznymi.

W publikacji 3. retrospektywnej ocenie poddano 87 pacjentów z lewostronnym, miejscowo zaawansowanym rakiem jelita grubego lub górnej odbytnicy, kwalifikowanych do radykalnego leczenia operacyjnego między styczniem 2014r. a grudniem 2015r. Kryteriami włączenia do badania były: a) rak dystalnej esicy, złącza esiczo-odbytniczego lub górnej odbytnicy (>10 cm od brzegu odbytu w kolonoskopii); b) miejscowo-zaawansowany charakter choroby, bez naciekania sąsiednich narządów; c) niestosowanie leczenia przedoperacyjnego. Kryteria wyłączenia z badania stanowiły: a) obecność współwystępujących nowotworów; b) zastosowanie chemio- i/lub radioterapii przedoperacyjnie; c) obecność zaburzeń hematologicznych lub innych stanów chorobowych mogących istotnie wpłynąć na wykładniki stanu zapalnego; d) stosowanie leczenia immunosupresyjnego w wywiadzie. Poddawano analizie morfologię krwi obwodowej pobraną przed leczeniem chirurgicznym; na jej podstawie wyliczano wartości LMR, NLR i PLR. Pooperacyjny materiał histopatologiczny oceniano immunohistochemicznie pod kątem nacieku limfocytów CD3+ i CD8+. Analizowano związki między parametrami LMR, NLR i PLR a gęstością limfocytów CD3+ i CD8+ w tkance guza, a także wartość prognostyczną markerów SIR i TILs.

Wyniki

W publikacji 1. w prospektywnie wyselekcjonowanej grupie 60 pacjentów było 43 mężczyzn i 17 kobiet. Mediana wieku wynosiła 66,5 lat (zakres 29-89 lat). Większość pacjentów miała chorobę nowotworową w stopniu zaawansowania IIIB i IIIC wg klasyfikacji TNM. U niemal połowy pacjentów (47%) stwierdzono raka niskiej odbytnicy (<5 cm od brzegu odbytu do dolnego brzegu guza), u 40% środkowej (5-10cm od brzegu odbytu), a u 13% górnej (>10cm od brzegu odbytu). W około połowie przypadków (47% pacjentów) wartość markera CEA mieściła się w granicach normy (<5 ng/mL). Współczynnik Kappa-Cohena zgodności między pomiarami dla LMR to 0,59 (95% CI, 0,39–0,79), dla NLR - 0,45 (95% CI, 0,22–0,68), a dla PLR - 0,53 (95% CI, 0,32–0,75), co oznacza, że we wszystkich trzech przypadkach zachodziła umiarkowana zgodność między pomiarami. Średnia procentowa zmiana między trzecim a pierwszym pomiarem poziomu limfocytów, monocytów, neutrocytów i trombocytów wahała się między -5,59% a 4,76%, zaś błąd standardowy między 2,0 a 3,9. Przyjęto punkty odcięcia dla niskich i wysokich wartości LMR, NLR i PLR na podstawie danych z literatury na poziomie odpowiednio 2,6, 3,0 i 150 (12, 14, 50, 51). W trzech pomiarach odsetek pacjentów należących do tej samej grupy (niskich lub wysokich wartości) wynosił dla LMR, NLR i PLR odpowiednio: 68,3%; 68,3% i 70%. Gdy wartość LMR w pierwszym pomiarze znajdowała się poza zakresem 2,2-3,0 (+/- 0,4 w stosunku do punktu odcięcia), ryzyko zakwalifikowania pacjenta do innej niż pierwotnie grupy (niskich lub wysokich wartości LMR) w drugim pomiarze wynosiło 5% (95% CI, 1,0–13,9%). W przypadku NLR, gdy wartość była poza zakresem 2,5-3,5 (+/- 0,5), ryzyko zmiany grup w drugim pomiarze wynosiło 8,3% (95% CI, 2,8–18,4%), zaś w przypadku PLR poza zakresem 125-175 (+/- 25) było to 10% (95% CI, 3,8–20,5%). Współczynniki korelacji między pierwszym a trzecim pomiarem wynosiły dla LMR 0,776 ($p < 0,00001$), dla NLR 0,696 ($p < 0,000089$), a dla PLR 0,751 ($p < 0,00001$). W analizie związków z parametrami kliniczno-patologicznymi zaobserwowano statystycznie znamienną zależność między wartością PLR a zajęciem regionalnych węzłów chłonnych ($p = 0,033$). Analiza post-hoc potwierdziła, że wartość PLR była niższa w grupie pacjentów bez zajęcia regionalnych węzłów chłonnych w stosunku do grupy pacjentów z zajęciem regionalnych węzłów chłonnych N1 i N2; odpowiednio: 116,35 (89,14–145,30); 147,27 (62,70–452,56); 164,41 (93,47–321,83). Wykazano znamienną pozytywną korelację między wartością LMR a naciekiem zapalnym w materiale histopatologicznym ($r = 0,38$, $p = 0,044$) i CPS ($r = 0,45$, $p = 0,016$). Nie stwierdzono wartości prognostycznej markerów LMR, NLR i PLR w 5-letniej obserwacji pacjentów.

W publikacji 2. spośród 371 pacjentów kryteria włączenia spełniło 184. Dodatni status EMVI stwierdzono u 78 pacjentów, a ujemny u 106. W EMVI-dodatniej populacji w porównaniu z EMVI-ujemną statystycznie częściej występowały duże guzy - T2 lub T3 (91% vs. 66%; $p < 0,001$) z zajęciem regionalnych węzłów chłonnych ($p < 0,001$); przekładało się to na większe zaawansowanie choroby wg klasyfikacji TNM ($p < 0,001$). Poziom neutrocytów, trombocytów i markera CEA był istotnie niższy w

grupie pacjentów EMVI-ujemnych w porównaniu z grupą EMVI-dodatnich (p = 0,041 dla neutrocytów, p < 0,001 dla trombocytów i p = 0,027 dla CEA). Wśród pacjentów pierwotnie EMVI-dodatnich obserwowano statystycznie znamiennie większe patologiczne zaawansowanie węzłowe (pN) (p < 0,001) i patologiczny stopień zaawansowania (p = 0,002) oraz nieznamiennie statystycznie większą wielkość guza (pT) (p = 0,078). W analizach statystycznych obejmujących krzywą ROC wszystkie parametry LMR, NLR i PLR okazały się słabymi wyznacznikami statusu EMVI (odpowiednio krzywa AUC= 0,49, 0,56 i 0,54). Nie stwierdzono korelacji między statusem EMVI a wartością markerów SIR.

W badaniu 3. kryteria włączenia spełniło 50 pacjentów. Po analizie histopatologicznej u 22% pacjentów stwierdzono chorobę w I, u 36% w II, a u 44% w III stopniu zaawansowania. Nie stwierdzono zależności między wartościami LMR, NLR, PLR lub gęstością limfocytów CD3+ i CD8+ w obrębie tkanki guza a stopniem zaawansowania choroby. Pacjentów podzielono na grupy z wysokimi i niskimi wartościami markerów SIR zgodnie z zasadami z poprzednich badań, punkty odcięcia ustalono na podstawie danych literaturowych. Stwierdzono znamienną statystycznie różnicę między poziomem limfocytów CD3+ w centrum guza między grupami pacjentów z niskimi i wysokimi poziomami NLR – odpowiednio 1767,86/mm2 (zakres: 705,36–3900,00/mm2) vs. 1233,93/mm2 (703,57–2950,00), (p = 0,044). Pacjentów poddano ponad 6-letniej obserwacji. W tym czasie 36% pacjentów zmarło. Pacjenci z wartościami LMR >2,6 mieli dłuższe OS w porównaniu z pacjentami z LMR ≤2,6 (p < 0,001). Pacjenci z wartościami NLR >3 mieli krótsze OS niż pacjenci z NLR ≤3 (p = 0,001). Pacjenci z wartościami PLR ≤ 150 mieli dłuższy OS w porównaniu z pacjentami z PLR >150, wynik nie był jednak statystycznie znamienny (p = 0,095). Nie stwierdzono korelacji między OS a gęstością limfocytów CD3 i CD8+ w mikrośrodowisku guza.

Wnioski

W oparciu o zbiór prac wysunięto następujące wnioski:

1. Zachodzi korelacja między obwodowymi i lokalnymi markerami CRI w populacji pacjentów z miejscowo zaawansowanym rakiem jelita grubego i odbytnicy. Wartość LMR jest związana z naciekiem zapalnym, a poziom NLR z gęstością limfocytów CD3+ w mikrośrodowisku guza.
2. Zachodzi korelacja między wartością LMR a obecnością komórek z ekspresją PD-L1 w mikrośrodowisku guza.
3. Powtarzalność markerów SIR jest umiarkowana w grupie pacjentów z miejscowo zaawansowanym rakiem odbytnicy.
4. Obecność EMVI jest związana z większym zaawansowaniem guza nowotworowego wg klasyfikacji TNM. Zachodzi zależność pomiędzy statusem EMVI a poziomem CEA,

neutrocytów i trombocytów. Nie zaobserwowano korelacji między markerami SIR a statusem EMVI.

5. Markery SIR wykazują wartość prognostyczną w populacji pacjentów z miejscowo zaawansowanym lewostronnym rakiem jelita grubego i górnej odbytnicy.
6. Nie stwierdzono prognostycznej wartości markerów SIR w populacji ograniczonej do pacjentów z miejscowo zaawansowanym rakiem odbytnicy.

Publikacje wchodzące w skład rozprawy doktorskiej

Publikacja 1.

"A Prospective Study on the roles of the Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) in Patients with Locally Advanced Rectal Cancer"

„Rola współczynnika limfocytów do monocytów (LMR), neutrocytów do limfocytów (NLR) i trombocytów do limfocytów (PLR) u pacjentów z miejscowo zaawansowanym rakiem odbytnicy w prospektywnym badaniu klinicznym"

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Celem pracy była analiza roli markerów SIR: LMR, NLR i PLR u pacjentów z LARC i ocena możliwości ich zastosowania w praktyce klinicznej. Badano powtarzalność markerów SIR, związki z czynnikami kliniczno-patologicznymi oraz wartość prognostyczną. Prospektywnej analizie poddano 60 wyselekcjonowanych pacjentów z miejscowo zaawansowanym rakiem odbytnicy leczonych w Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie − Państwowym Instytucie Badawczym w Warszawie między sierpniem 2017r. a grudniem 2020r. Do badania włączono pacjentów, którzy zostali zakwalifikowani decyzją zespołu wielodyscyplinarnego do neoadjuwantowej radio/radiochemioterapii. Wyłączeni z badania byli pacjenci ze współwystępującymi nowotworami litymi, po leczeniu immunosupresyjnym, z ostrym lub przewlekłym stanem zapalnym, nowotworami hematologicznymi, chorobami autoimmunologicznymi i innymi stanami medycznymi mogącymi istotnie wpływać na zaburzenia wykładników stanu zapalnego. U wszystkich pacjentów wykonano trzy badania morfologii krwi przed rozpoczęciem leczenia onkologicznego. Na podstawie wyników oceniano poziom LMR, NLR i PLR. Resekcję guza nowotworowego przeprowadzono u 44 pacjentów. Następnie poddano analizie pooperacyjny materiał histopatologiczny. Stwierdzono znamienną statystycznie korelację między LMR a naciekiem zapalnym w mikrośrodowisku guza oraz ekspresją PD-L1 na komórkach nowotworowych, limfocytach i makrofagach (CPS). Wartość PLR była istotnie statystycznie powiązana z zajęciem regionalnych węzłów chłonnych. Markery SIR okazały się umiarkowanie powtarzalne. Nie stwierdzono prognostycznej wartości markerów SIR w odniesieniu do OS w grupie pacjentów z LARC.

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A Prospective Study on the Roles of the Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), and Platelet-to-Lymphocyte Ratio (PLR) in Patients with Locally Advanced Rectal Cancer

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Abstract: Rectal cancer constitutes over one-third of all colorectal cancers (CRCs) and is one of the leading causes of cancer-related deaths in developed countries. In order to identify high-risk patients and better adjust therapies, new markers are needed. Systemic inflammatory response (SIR) markers such as LMR, NLR, and PLR have proven to be highly prognostic in many malignancies, including CRC; however, their roles in locally advanced rectal cancer (LARC) are conflicting and lack proper validation. Sixty well-selected patients with LARC treated at the Maria Sklodowska-Curie National Research Institute of Oncology in Warsaw, Poland, between August 2017 and December 2020 were prospectively enrolled in this study. The reproducibility of the pre-treatment levels of the SIR markers, their correlations with clinicopathological characteristics, and their prognostic value were evaluated. There was a significant positive correlation between LMR and cancer-related inflammatory infiltrate (r = 0.38, p = 0.044) and PD-L1 expression in tumor cells, lymphocytes, and macrophages (combined positive score (CPS)) (r = 0.45, p = 0.016). The PLR level was correlated with nodal involvement (p = 0.033). The SIR markers proved to be only moderately reproducible and had no significant prognostic value. In conclusion, the LMR was associated with local cancer-related inflammation and PD-L1 expression in tumor microenvironments. The validity of SIR indices as biomarkers in LARC requires further investigation.

Keywords: LMR (lymphocyte-to-monocyte ratio); NLR (neutrophil-to-lymphocyte ratio); PLR (platelet-to-lymphocyte ratio); inflammatory infiltrate; CPS (combined positive score); rectal cancer

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1. Introduction

Rectal cancer constitutes approximately 35% of all colorectal cancers (CRCs). Its incidence in the European Union is estimated at 125,000 per year, and this is predicted to rise due to sociodemographic changes [1,2]. An alarming increase in the incidence of both colon and rectal cancers in young adults has been observed in recent years [3,4]. Prognoses, especially in advanced stages of the disease, remain unsatisfactory [5]. The current standard of care for patients with locally advanced rectal cancer (LARC) is neoadjuvant radiotherapy/chemoradiotherapy followed by surgery according to total mesorectal excision (TME) principles with or without postoperative chemotherapy [6–8]. However, the

impact of such an approach on overall survival (OS) remains unclear, and it may cause long-term toxicities and impaired quality of life [9,10]. New markers are required to appropriately identify low- and high-risk patients, which is crucial for properly adjusting patients' therapy. Blood-based systemic inflammatory response (SIR) markers such as LMR, NLR, and PLR are simple and cheap biomarkers with proven prognostic value in CRC [11–14]. However, the proper validation of these markers is lacking, and their roles in LARC are uncertain [15,16]. We conducted a prospective study on a well-selected group of patients with LARC. We investigated the reproducibility of the SIR markers, their correlations with clinicopathological characteristics, and their prognostic value.

2. Materials and Methods

A single-arm prospective study among patients treated at the Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw was conducted. The eligibility criteria were as follows: (1) the patients were diagnosed with primary locally advanced rectal cancer confirmed by histopathology; (2) their clinical records, including demographic data and laboratory data, were available and complete; (3) the performance statuses of the patients were ECOG 0-2, and the patients had qualified to receive radio/chemoradiotherapy by multidisciplinary teams; and (4) the patients were >18 years old. The exclusion criteria were as follows: (1) the presence of distant metastasis at the time of diagnosis; (2) the presence of malignant tumors in other organs; (3) the presence of acute or chronic inflammatory diseases, hematological malignancies, autoimmune diseases, and other medical conditions that could affect inflammatory markers; and (4) prior immunosuppressive therapy. Blood samples from the patients were obtained three times within a median period of 21 days (range of 7–55 days). All the tests were performed prior to any oncological treatments. The differential white blood cell counts were analyzed using a Sysmex XN-550 hematology analyzer following the manufacturer's protocol. The LMR, NLR, and PLR were calculated from the blood samples by dividing an absolute lymphocyte count by an absolute monocyte count, an absolute neutrophil count by an absolute lymphocyte count, and an absolute platelet count by an absolute lymphocyte count, respectively. The patients were divided in terms of the baseline values of their SIR markers into high and low LMR, NLR, and PLR groups. The cut-off values were determined based on our previous studies and the data available in the literature [17–20].

Formulas:

LMR—absolute lymphocyte count (g/L)/absolute monocyte count (g/L)

NLR—absolute neutrophil count (g/L)/absolute lymphocyte count (g/L)

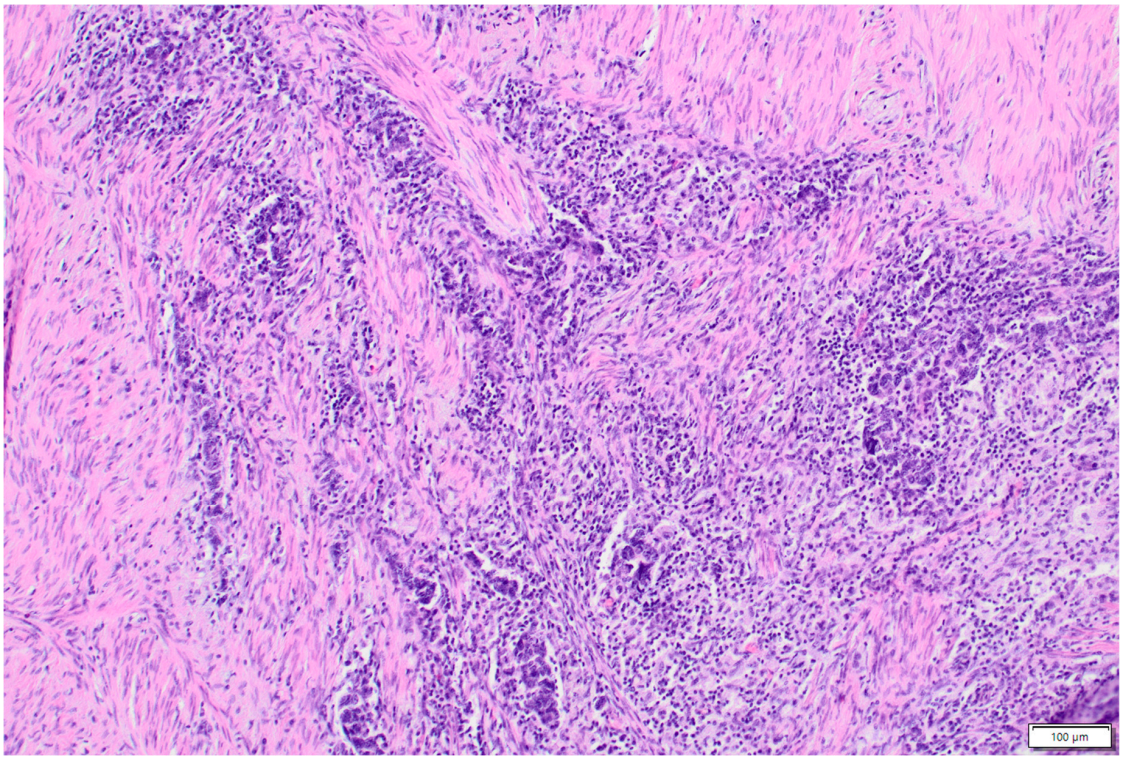
PLR—absolute platelet count (g/L)/absolute lymphocyte count (g/L)

All the patients received neoadjuvant radio/chemoradiotherapy according to the multidisciplinary teams' decisions, which were based on the stage of the disease. Ten patients did not agree to proceed with surgery. Six patients progressed/proved to be inoperable before surgery. Surgery was performed on 44 patients.

2.1. Histopathological Analysis

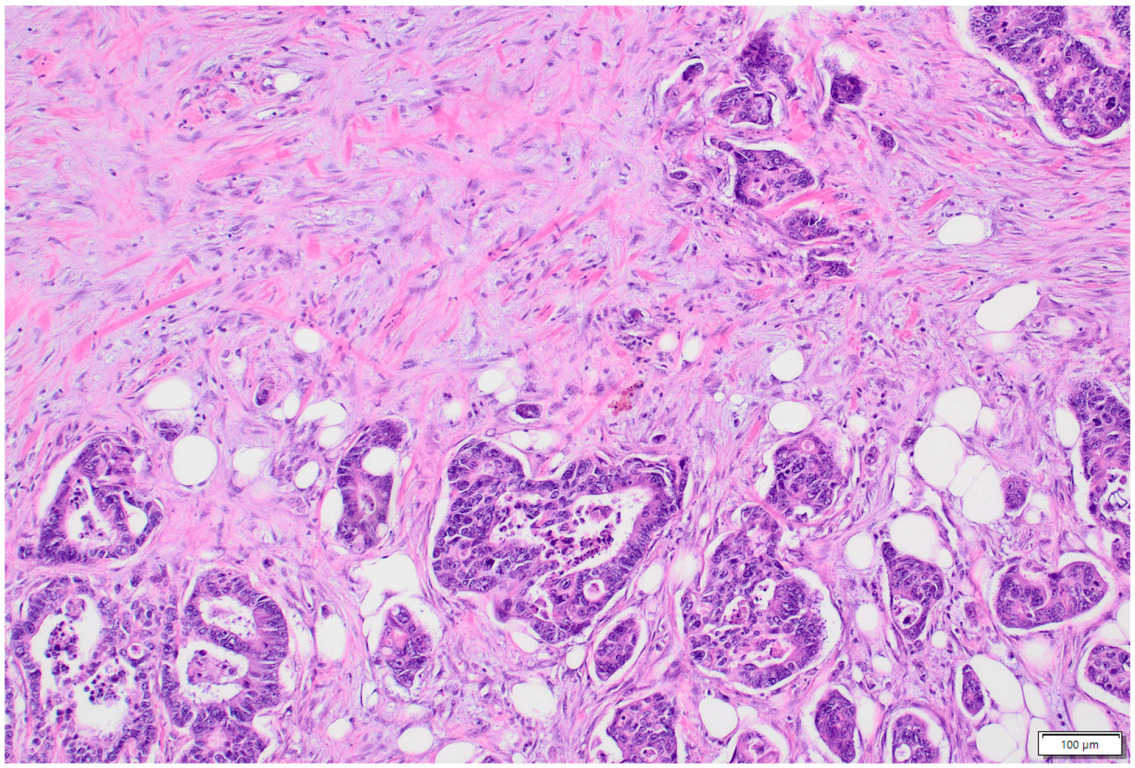
The post-surgical pathological results were collected and analyzed. There were 10 cases of complete pathological response (pCR). In two cases, no pathological specimens were available after surgery, and in three cases, the specimens were deemed not suitable for the histopathological analysis. Twenty-nine specimens were found suitable for the analysis. The presence of tumor-infiltrating immune cells in the tumor centers and the invasive margins was evaluated by immunohistochemistry using the antibodies for the CD8 antigen. For the immunohistochemical staining, primary monoclonal antibodies against CD8 (DAKO, Glostrup, Denmark, Cat. No IR623) with a DAKO EnVision FLEX detection sys-

tem (DAKO, Denmark, Cat. No K8002) were used. Paraffin sections (4 µm on silanized slides) were deparaffinized, rehydrated, and then stained according to the manufacturer's procedures. In a semi-quantitative assessment, a four-digit scale (0: 0–10% of the area of scarce and mild staining, 1: 11–50% of the area of moderate or intensive staining, 2: 50–75% of the area of intermediate or intensive staining, and 3: >75% of the area of intermediate or intensive staining) of the density of lymphocytes was used in the measurements for the tumor invasive margins. The inflammatory infiltrates containing lymphocytes, plasmacytes, monocytes/macrophages, and neutrophils were assessed histologically on H&E basic stain at the invasive fronts of the tumors using the same semi-quantitative four-digit scale. An example of intensive inflammatory infiltrates and scarce inflammatory infiltrates at the invasive margins is presented in Figure 1. Primary antibodies against MSH6 (DAKO, Denmark, Cat. No IR086) and PMS2 (DAKO, Denmark IR087) were used to detect the expression of microinstability indicator proteins. The percentage of positive cancer cells was estimated in each case, and the internal positive control consisted of lamina propria inflammatory cells and/or nontumoral glandular cells. As for the PD-L1 expression, clone 22C3 of the monoclonal antibody (DAKO, Denmark, Cat. No SK006) was used, and the staining was performed automatically in a closed system as supplied by the manufacturer. The expression was calculated as a CPS given the number of the PD-L1-staining cells (tumor cells, lymphocytes, macrophages) relative to all viable tumor cells, multiplied by 100% (the range of the results was between 0 and 100). An example of high and low expression of PD-L1-staining cells is presented in Figure 2.



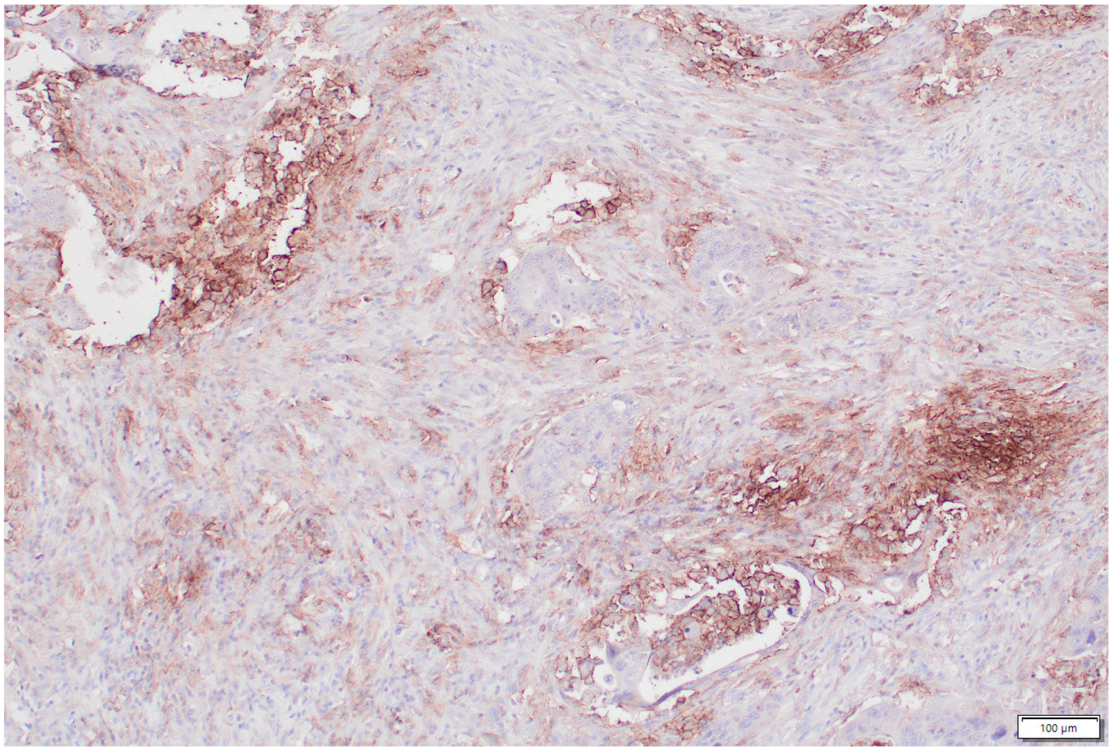
(**A**)

Figure 1. *Cont.*



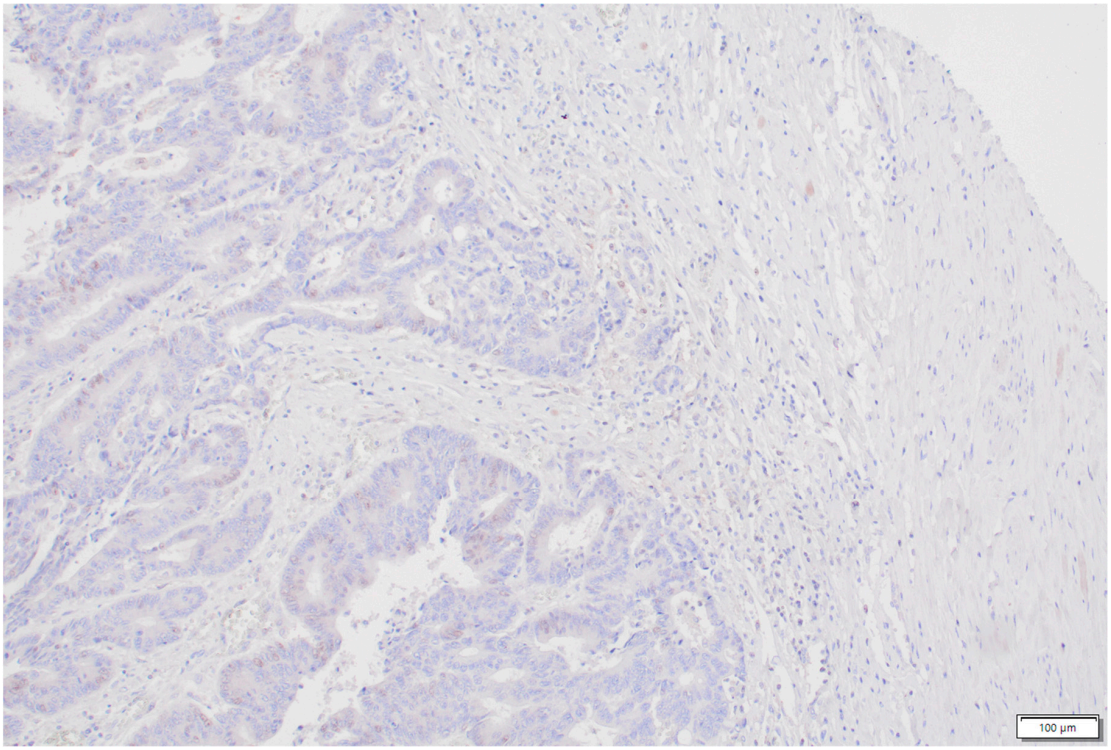
(**B**)

Figure 1. Inflammatory infiltrates at the invasive margins of the cancers. The intensive inflammatory infiltrate (**A**) versus nearly no inflammatory cells (**B**) at the invasive margins of the tumors (both H&E ×100).



(**A**)

Figure 2. *Cont.*



(**B**)

Figure 2. PD-L1-staining cells at the invasive margins of the cancers. The high expression of PD-L1-staining cells (**A**) versus nearly no PD-L1-staining cells (**B**) at the invasive margins of the tumors (DAKO 22C3 antibody).

2.2. Statistical Analysis

The Shapiro–Wilk test was used to test the normality of the data distribution. The analysis of the repeatability of the measurements of SIR markers was evaluated using the Friedman test. Binomial variables were compared between measurements with the McNemar test. Additionally, confidence intervals for the proportions were calculated using a binomial exact calculation. Cohen's Kappa was calculated to assess the extent of agreement between the first and the second measurements, including 95% confidence intervals. The relationships between parameters were assessed using Pearson's correlation analysis. Statistical analyses were performed using the IBM SPSS Statistics ver. 23 software package and R software, version 4.0.5. The Kaplan–Meier procedure was performed to compare the survival and time without relapse between patients, with low and high levels of the LMR, NLR, and PLR. The log-rank test was used to verify whether any significant differences between groups were present. The 95% confidence intervals were calculated for a cumulative proportion of the patients who did not die/relapse. Correlations between qualitative or semi-qualitative variables were verified using Spearman's correlation coefficients. The levels of the LMR, NLR, and PLR vs. the T, N, CR, and presence of progression were analyzed using Mann–Whitney U tests (comparison of 2 groups) or with a Kruskal–Wallis test (comparison of 3 groups), with a Dunn post hoc test.

2.3. Ethical Considerations

The study conformed to the provisions of the Declaration of Helsinki and was approved by the ethics committee of the National Institute of Oncology. All patients were informed of the investigational nature of this study and provided written informed consent.

3. Results

A total of 60 patients with rectal cancer treated at the Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw between August 2017 and December 2020 were prospectively enrolled in the study. Forty-three males and seventeen females were included. The median age was 66.5 years (range of 29–89 years old). All the patients in the study were citizens of Poland of Caucasian ethnicity. The distributions of the cancer stages were as follows: stages II–IIIA, 8 (13%); stage IIIB, 41 (68%); and stage IIIC, 10 (17%). The stage of one of the patients remained undefined. There were no stage I or stage IV patients. All the rectal cancers were adenocarcinomas. The intermediate differentiation of the tumor was the most common—in 42 (70%) patients followed by the undefined differentiation—14 (23.3%). Two (3.3%) rectal cancers were well-differentiated (G1) and two (3.3%) poorly differentiated (G3). In terms of localization of the tumor within the rectum (distance of the lowest portion of the tumor from the anal verge), 28 (47%) patients had low, 24 (40%) middle, and 8 (13%) high rectal cancer. There were 15 (25%) smokers and 45 (75%) non-smokers. Most of the patients were overweight—23 (38%); 19 (32%) had normal weight; 17 (28%) were obese, and only 1 (2%) patient was underweight. Almost half of the patients (47%) had normal levels of carcinoembryionic antigen (<5.0 ng/mL). The characteristics of the patients are presented in Table 1.

Table 1. Characteristics of the patients.

	All Patients (*n* = 60)
Age (years), median (range)	66.5 (29–89)
Sex, *n* (%)	
Male	43 (71.7)
Female	17 (28.3)
BMI, *n* (%)	-
<18.5	1 (2)
18.5–25	19 (32)
25–30	23 (38)
≥30	17 (28)
Smokers, *n* (%)	15 (25)
Non-smokers, *n* (%)	45 (75)
CEA (ng/mL), median (range)	21.89 (0.86–69.96)
Normal level (<5.0 ng/mL), n (%)	28 (47)
Elevated level (≥5.0 ng/mL), n (%)	32 (53)
Tumor, *n* (%)	
T3	55 (91.7)
T4	5 (8.3)
Lymph nodes, *n* (%)	
N0	8 (13.3)
N1	35 (58.3)
N2	16 (26.7)
Nx	1 (1.7)

Table 1. *Cont.*

	All Patients (*n* = 60)
Grade, *n* (%)	
G1	2 (3.3)
G2	42 (70)
G3	2 (3.3)
Gx	14 (23.3)
Stage, *n* (%)	
II–IIIA	8 (13.3)
IIIB	41 (68.3)
IIIC	10 (16.7)
Tumor localization, *n* (%)	
Low rectum	28 (47)
Middle rectum	24 (40)
High rectum	8 (13)
Time between measurements (days), median (range)	
1st–2nd	9 (1–42)
2nd–3rd	11 (1–34)
1st–3rd	21 (7–55)

BMI, body mass index; CEA, carcinoembryonic antigen.

The median values of the lymphocytes, monocytes, neutrophils, and platelet counts, as well as their ratios, are shown in Table A1.

3.1. Reproducibility

The patients were divided into high and low groups according to the baseline values of each SIR marker. The predetermined cut-offs were 2.6 for the LMR, 3.0 for the NLR, and 150 for the PLR. The numbers of patients who belonged to each group in each measurement are presented in Table A2.

Over half of the patients (56.7%) (95% CI, 43.2–69.4%) were classified as LMR high, and 61.7% (95% CI, 48.2–73.9%) and 51.7% (95% CI, 38.4–64.8%) of the patients were assigned to the NLR low and PLR low groups accordingly. After the second measurements, 81.7% (95% CI, 69.6–90.5%) of the patients belonged to the same groups (LMR high or LMR low). In terms of the NLR and PLR, 73.3% (95% CI, 60.3–83.9%) and 78.3% (95% CI, 65.8–87.9%) of the patients were in the same groups, respectively. After three measurements, the percentages of patients who stayed in the same groups were nearly identical, as follows: 68.3% (95% CI, 55.0–79.7%) for the LMR and NLR and 70.0% (95% CI, 56.8–81.2%) for the PLR. For the LMR, NLR, and PLR, there were no significant changes in the percentages of the patients classified as low or high between all three measurements ($p > 0.05$ in all comparisons). The mean percentage change between the third and the first measurements of the lymphocytes, monocytes, neutrophils, and platelet counts ranged from −5.59% to 4.76%, and the standard errors ranged from 2.0 to 3.9 (Table 2).

The Cohen's Kappa statistic for the extent of the agreement between the first and second measurements for the LMR was $\kappa = 0.59$ (95% CI, 0.39–0.79) ($p < 0.001$). For the NLR, the Kappa was $\kappa = 0.45$ (95% CI, 0.22–0.68) ($p < 0.001$), and for the PLR, $\kappa = 0.53$ (95% CI, 0.32–0.75) ($p < 0.001$), meaning in all cases, there was a moderate agreement between both measurements.

Table 2. Calculations of the percentages of the changes between the third measurements vs. the first measurements.

% Change	*n*	Mean	Standard Deviation	Standard Error	Median	Minimum	Maximum
L	60	4.76	30.23	3.9	0.75	−60.61	92.86
M	60	3.88	24.39	3.1	4.78	−40.00	85.71
N	60	−5.59	20.57	2.7	−8.20	−47.70	43.66
WBC	60	−2.39	17.28	2.2	−3.86	−39.78	42.12
PLT	60	1.29	15.30	2.0	−0.70	−29.32	44.60

L, lymphocytes; M, monocytes; N, neutrophils; WBC, white blood cells; PLT, platelets.

If the LMR at the first measurement was out of the range of 2.2–3.0 (±0.4 from the cut-off), then the risk of misclassification in the second measurement, defined as an affiliation to a different (high or low) group than initially, dropped to 5.0% (95% CI, 1.0–13.9%). In the case of the NLR, when it was outside of the range of 2.5–3.5 (±0.5) in the first test, it was 8.3% (95% CI, 2.8–18.4%), and in the case of a PLR outside of the range of 125–175 (±25), it was 10.0% (95% CI, 3.8–20.5%).

An analysis of the correlation between the first and third measurements of the LMR, NLR, and PLR was conducted. The LMR values were correlated with a coefficient of 0.776 ($p < 0.00001$). The NLR and PLR were correlated with coefficients of 0.696 ($p < 0.000089$) and 0.751 ($p < 0.00001$), respectively (Figure A1).

3.2. Correlation with Clinicopathological Characteristics

There was no significant correlation between the LMR, NLR, and PLR and the tumor size. There were no relationships between the pre-treatment levels of the SIR markers and both the progression and inoperability after neoadjuvant therapy as well as complete pathological responses. There were significant differences in the PLR levels between the N0, N1, and N2 subgroups ($p = 0.033$). A post hoc analysis confirmed that the PLR level in the N0 group was lower (116.35 (89.14–145.30) vs. N1, 147.27 (62.70–452.56); and vs. N2, 164.41 (93.47–321.83). There was no correlation between the LMR and the NLR, and the nodal involvement was observed (Table 3).

Table 3. Average value of the LMR, NLR, and PLR depending on the size of the tumor, nodal status, complete pathological response, and presence of progression after neoadjuvant treatment.

	LMR Avg *		NLR Avg *		PLR Avg *	
	Median (Range)	*p*-Value	Median (Range)	*p*-Value	Median (Range)	*p*-Value
T						
T3	2.94 (1.12–6.91)	0.470	2.71 (1.01–6.90)	0.336	142.46 (62.70–452.56)	0.377
T4	2.49 (1.19–3.84)		3.10 (2.07–8.92)		185.88 (97.58–390.89)	
N						
0	3.52 (1.50–5.34)	0.714	2.27 (2.06–4.07)	0.457	116.35 (89.14–145.30)	0.033 (0 vs. 1 and 2)
1	2.91 (1.14–6.91)		2.91 (1.01–8.92)		147.27 (62.70–452.56)	
2	147.27 (62.70–452.56)		2.68 (1.94–5.44)		164.41 (93.47–321.83)	
CR						
No pCR	3.02 (1.12–6.91)	0.867	2.71 (1.01–6.90)	0.796	136.65 (62.70–392.60)	0.309
pCR	2.85 (1.80–5.23)		2.56 (1.91–6.39)		153.55 (93.47–452.56)	

Table 3. *Cont.*

	LMR Avg *		NLR Avg *		PLR Avg *	
	Median (Range)	***p*-Value**	**Median (Range)**	***p*-Value**	**Median (Range)**	***p*-Value**
Progression/inoperability						
No	2.86 (1.12–6.91)	0.990	2.71 (1.01–8.92)	0.805	144.43 (62.70–452.56)	0.931
Yes	2.98 (1.14–3.86)		2.72 (1.76–6.15)		149.28 (95.91–349.34)	

LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; pCR, pathological complete response; *, average level from all three measurements with analyses using the Mann–Whitney U test (T; progression/inoperability, CR) or the Kruskal–Wallis test (N).

There was no significant correlation between the LMR, NLR, and PLR and the pre-treatment level of CEA ($p > 0.05$ in all cases) (Table A3). There was a significant positive correlation between the LMR and the cancer-related inflammatory infiltrates in the resected tissues (r = 0.38, $p = 0.044$) and the PD-L1 expression in the tumor cells and tumor-associated leukocytes (CPS) (r = 0.45, $p = 0.016$). The NLR and PLR were not related to the level of CPS or the inflammatory infiltrates. The correlation between the density of the CD8+ lymphocytes and the LMR, PLR, and NLR was not significant (Table 4).

Table 4. Correlation between the LMR, NLR, and PLR and the CPS, CD8+ lymphocytes, and inflammatory infiltrates.

	CPS		CD8+		Inflammatory Infiltrate	
	R	***p* Value**	**R**	***p* Value**	**r**	***p* Value**
LMR avg *	0.45	**0.016**	0.21	0.266	0.38	0.044
NLR avg *	−0.19	0.316	−0.08	0.691	−0.15	0.447
PLR avg *	0.16	0.401	0.06	0.744	0.09	0.626

LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CPS, combined positive score; *, average level from all three measurements; r, Spearman's correlation coefficient.

The combined positive score was significantly positively correlated with the CD8+ (r = 0.56, $p = 0.002$), as well as with the inflammatory infiltrates (r = 0.51, $p = 0.005$) (Table A4). There was only one case of mismatch repair deficiency among the twenty-nine histopathologically assessed specimens (3.45%).

3.3. Prognostic Value

The population of patients was analyzed in terms of recurrence-free survival (RFS) and OS depending on the pre-treatment levels of the LMR, NLR, and PLR.

3.4. Lymphocyte-to-Monocyte Ratio

The cumulative proportion of patients who did not relapse at the end of the observation period was 32% (95% CI = 8%; 100%) for the low LMR level group and 68% (95% CI = 53%; 87%) for the high LMR level group. The mean number of months without relapse was M = 39.03 for the low LMR level group and M = 47.01 for the high LMR level group ($p = 0.641$). At the end of the observation period, the cumulative proportion of alive patients was 80% (95% CI = 65%; 97%) for the low LMR level group and 80% (95% CI = 66%; 99%) for the high LMR level group. The mean time of survival was M = 44.81 months for the subjects with low LMR levels and M = 52.61 months for the subjects with high LMR levels ($p = 0.597$) (Figure 3).

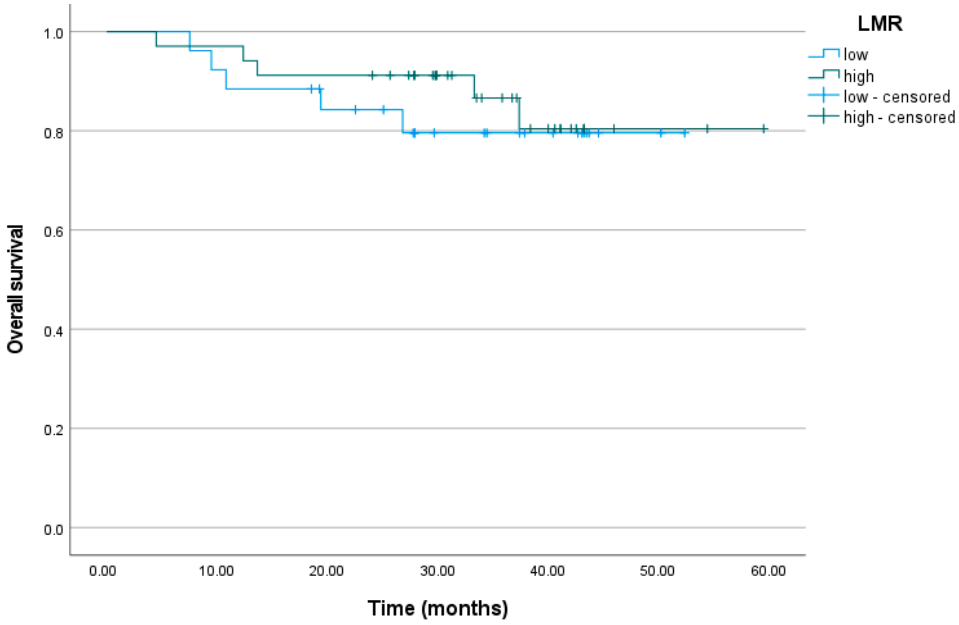


Figure 3. Overall survival curve for the patients with low and high LMR levels. LMR, lymphocyte-to-monocyte ratio.

3.5. Neutrophil-to-Lymphocyte Ratio

The mean number of months without relapse for the patients with low NLR levels was M = 48.79, and for the patients with high NLR levels, it was M = 36.91. The cumulative proportion of subjects who did not relapse at the end of the observation period was 71% (95% CI = 57%; 90%) for the low NLR level group and 30% (95% CI = 7%; 100%) for the high NLR level group (p = 0.225). No differences were detected between the survival times of the patients with low and high NLR levels (p = 0.927). The mean time of survival was M = 51.36 months for the subjects with low NLR levels and M = 45.66 months for the subjects with high NLR levels. The cumulative proportion of alive patients at the end of the follow-up period was 76% (95% CI = 59%; 98%) for the low NLR level group and 83% (95% CI = 69%; 100%) for the high NLR level group (Figure 4).

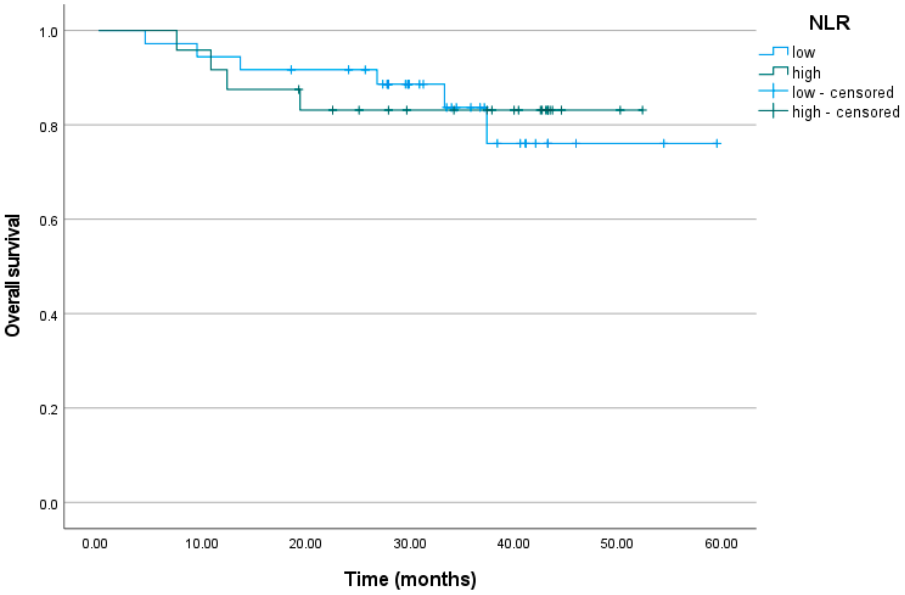


Figure 4. Overall survival curve for the patients with low and high NLR levels. NLR, neutrophil-to-lymphocyte ratio.

3.6. Platelet-to-Lymphocyte Ratio

The cumulative proportion of patients who did not relapse at the end of the observation period was 63% (95% CI = 46%; 86%) for the low PLR level group and 47% (95% CI = 20%; 100%) for the high PLR level group. The mean number of months without a relapse was M = 40.86 among the patients with low PLR levels and M = 44.48 among the patients with high PLR levels (p = 0.869). The mean time of survival was M = 42.57 months for the patients with low PLR levels and M = 54.56 for the patients with high PLR levels. The cumulative proportion of alive subjects was 72% (95% CI = 56%; 94%) for the low PLR level group and 89% (95% CI = 78%; 100%) for the high PLR level group (p = 0.261) (Figure 5).

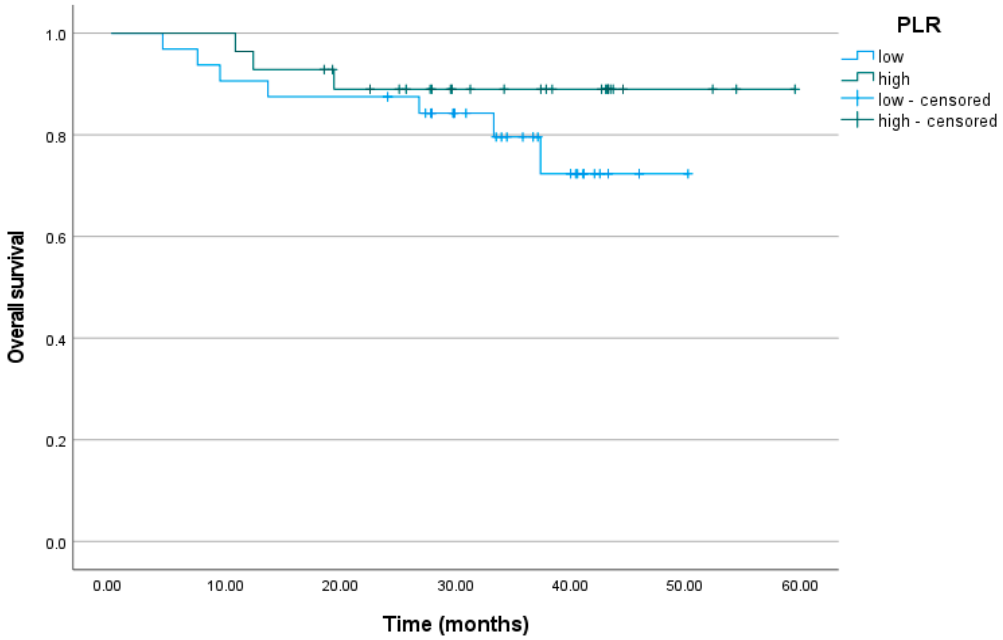


Figure 5. Overall survival curve for the patients with low and high PLR levels. PLR, platelet-to-lymphocyte ratio.

4. Discussion

Cancer may induce both local and systemic inflammatory reactions [21]. The LMR, NLR, and PLR are blood-based biomarkers of cancer-related inflammation. In our study, we proved that there was a strong correlation between the LMR and cancer-related inflammatory infiltrates in the resected tissues. Similar results have been reported for cholangiocarcinoma, colorectal, and breast cancers [22–24]. However, no correlation between the SIR markers and tumor-infiltrating CD8 lymphocytes was found, which was in line with other studies on both rectal and left-sided colon cancers [25,26]. This apparent discrepancy may have been due to the large populations of neutrophils, macrophages, or other subsets of lymphocytes in the inflammatory infiltrates. We found a correlation between the LMR and PD-L1 expression in the tumor cells and tumor-associated leukocytes relative to all the viable tumor cells (CPS). To our knowledge, these are the first data on a correlation between the SIR markers and CPS in colorectal cancer. In other malignancies, the data on correlations between SIR markers and PD-L1 expression are conflicting [27,28]. We found no association between the SIR markers and the level of CEA, which corresponded to a retrospective study on rectal cancer patients [29]. The PD-L1 expression in the immune cells was positively correlated with both the inflammatory infiltrates and the tumor-infiltrating CD8 lymphocytes. Similar relationships have been reported in hepatocellular carcinoma, cholangiocarcinoma, and colorectal cancer [30–32]. The LMR, NLR, and PLR are biomarkers with high prognostic value in many malignancies. However, their roles in LARC are not clear and lack proper validation. The number of studies assessing their reproducibility is very limited. To the best of our knowledge, our study is the first to directly investigate

this subject in a prospectively enrolled cohort. Reference and cut-off values for the SIR markers are not well-established. According to analyses of ostensibly healthy populations, the average values of the LMR, NLR, and PLR may differ depending on race, sex, and age. The mean values for the LMR in healthy individuals were significantly higher, and the mean values for the NLR and PLR were lower in comparison to our results [33–35]. Our findings were based on a well-selected group of patients with untreated LARC with no concomitant acute or chronic diseases that could have influenced the levels of inflammatory markers, which suggests that all three SIR markers are only moderately reproducible. When divided into high and low groups, the percentages of patients who stayed in the same groups after three measurements were nearly the same for all the parameters (68.3% for the LMR and NLR and 70% for the PLR). Nearly one-third of the patients' affiliations with a group changed between the assessments. However, if the first measurement was out of the range of approximately ±15% from the cut-off, the risk of misclassification in the second measurement dropped significantly, and in terms of the LMR, this dropped to 5% (95% CI, 1.0–13.9%), while for the NLR, it dropped to 8.3% (95% CI, 2.8–18.4%), and for the PLR, it dropped to 10% (95% CI, 3.8–20.5%). These results were in line with our previous retrospective study on the reproducibility of the LMR in patients with LARC, where two peripheral blood tests within five weeks prior to beginning anti-cancer therapies were performed [20]. The stability of the NLR over time, up to 100 days, has been demonstrated in cardiac surgery patients; however, it has not been confirmed in a cancer population [36]. No other studies investigating the reproducibility of SIR markers have been found in the literature. We analyzed the RFS and OS of patients depending on the levels of their LMR, NLR, and PLR. We found no statistically significant correlations in terms of RFS and OS between the high and low LMR, NLR, and PLR groups. These results were not consistent with the majority of studies assessing the whole population of CRC [37–40]. However, among trials restricted to LARC, the impacts of SIR markers on recurrences and survival have been conflicting. Wu et al. showed no correlation between the LMR and the DFS or OS in a non-metastatic rectal cancer population [15]. Similarly, in a large study of over 1500 LARC patients by Dudani et al., no statistically significant correlation between the NLR, PLR, and DFS and the OS was proven [16]. These findings were supported by the results of the study by Ishikawa and Portale et al. [41,42]. Most meta-analyses have suggested that the SIR markers in CRC have prognostic value, and these have included patients with both metastatic and non-metastatic disease [43,44]. The association between SIR markers and prognosis was less noted in non-metastatic stages. There are data that have indicated that SIR markers are associated with adverse OS in colon cancer but not in rectal cancer [45]. Our results confirmed that the prognostic value of the SIR markers in LARC is less evident than those among the whole CRC population. The phenomenon of cancer-related inflammation is important for understanding the roles of SIR markers. The relationship between cancer and inflammation has been investigated since the 19th century when Virchow first observed that cancer tends to originate from chronically inflamed sites [46]. Through the recruitment of inflammatory cells and cytokines, the production of reactive oxygen species, and the inhibition of repair programs, inflammation promotes the uncontrolled proliferation of defective cells and potentiates neoplastic risk. Inflammatory cells are abundant in a tumor's microenvironment [47]. They reflect a reaction of the host towards a tumor, but they also serve as a product of cancer-related cells and a tumor's predisposition toward invading and suppressing the immune system [48]. Lymphocyte counts reflect systemic inflammatory responses by inducing the production of anti-tumor cytokines, and cytotoxic activity suppresses a cancer's proliferation and spread [49]. Monocytes, on the contrary, have proven to contribute to a tumor's progression and metastatic activity [50]. Neutrophils, accounting for 50–70% of leukocytes, play a central role in cancer-related inflammation. Releasing reactive oxygen and nitrogen species that damage DNA, they play a substantial role in cancer initiation [51]. Tumor progression is boosted by neutrophil-derived chemokines and cytokines that mediate the process of angiogenesis [52]. Neutrocytes take part in suppressing T-lymphocyte proliferation, reducing the anti-tumoral

effect of NK cells and promoting metastatic spread [53,54]. Similarly, platelets, by releasing cytokines and growth factors, contribute to carcinogenesis. There is a substantial interaction between thrombocyte activation and cancer progression. Tumor cells produce cytokines, such as IL-6, that stimulate thrombocytosis. In turn, thrombocytes promote further tumor growth, leading to an even more intensive stimulation and activation of platelets [55]. These immunological interactions have led to the introduction of SIR markers and the investigation of their potential roles in clinical practice. Our study revealed interesting aspects of the SIR markers in LARC.

There were two main limitations concerning our study: (a) it had a relatively small group of patients, and (b) our studied population was homogenous, consisting entirely of Caucasian citizens of Poland. Moreover, other factors might have had an impact on the results of our study such as the lack of well-defined cut-off values for the LMR, NLR, and PLR and the possible influence of other parameters (e.g., age, sex, comorbidities, smoking) on the level of the LMR, NLR, and PLR. The time between measurements of blood samples varied, which might have had an impact on the blood results. Finally, the immunohistochemical data may suffer a bias due to the fact that several patients either did not proceed with surgery or had complete pathological responses. Future studies should include larger and mixed populations to confirm our results. Despite its limitations, our study explored subjects that are rarely present in the literature. A better understanding of the roles of SIR indices in LARC and their relationships with other clinicopathological features may enable the application of these markers in clinical practice.

5. Conclusions

The LMR, NLR, and PLR are peripheral blood-based markers of cancer-related inflammation. Our results suggest that the LMR is correlated with inflammatory infiltrates and PD-L1 expression in a tumor's microenvironment. However, the prognostic value of the SIR markers appears to be less evident among the patients with LARC compared to other colon cancers and most other malignancies, with no statistically significant impact on the RFS or OS in our study. The reproducibility of the SIR markers is moderate. More prospective studies are required to assess the validity of the SIR indices as biomarkers in LARC.

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Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from the patients for publishing this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to medical data privacy issues.

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Conflicts of Interest: The authors declare no competing interest.

Appendix A

Table A1. Median and mean values of the ALC, AMC, ANC, platelets, LMR, NLR, and PLR in three measurements.

X	First Measurement	Second Measurement	Third Measurement	All Measurements (Mean)	*p*-Value [1]
ALC (10^9/L), median (range)	1.72 (0.70–3.79)	1.65 (0.69–4.02)	1.67 (0.52–3.92)	1.67 (0.52–4.02)	0.541
ALC (10^9/L), mean (SD)	1.84 (0.69)	1.87 (0.76)	1.86 (0.73)	1.86 (0.69)	
AMC (10^9/L), median (range)	0.61 (0.30–1.30)	0.64 (0.27–5.26)	0.64 (0.33–1.21)	0.63 (0.27–5.26)	0.800
AMC (10^9/L), mean (SD)	0.68 (0.25)	0.77 (0.65)	0.68 (0.24)	0.71 (0.30)	
ANC (10^9/L), median (range)	4.89 (2.42–12.36)	5.10 (2.11–12.27)	4.43 (2.35–13.69)	4.84 (2.11–13.69)	0.770
ANC (10^9/L), mean (SD)	5.43 (2.05)	5.26 (2.00)	5.02 (2.03)	5.24 (1.91)	
Platelets (10^9/L), median (range)	273.00 (116.00–666.00)	254.00 (149.00–607.00)	269.00 (152.00–601.00)	264.00 (116.00–666.00)	0.198
Platelets (10^9/L), mean (SD)	293.00 (109.60)	284.00 (94.12)	291.00 (101.82)	289.00 (99.16)	
LMR, median (range)	2.71 (1.11–6.42)	2.95 (0.24–7.32)	2.8 (0.87–6.98)	2.89 (0.24–7.32)	0.766
LMR, mean (SD)	2.94 (1.22)	2.93 (1.25)	2.91 (1.18)	2.93 (1.11)	
NLR, median (range)	2.71 (1.17–7.58)	2.84 (0.81–8.96)	2.47 (1.04–10.22)	2.65 (0.81–10.22)	0.344
NLR, mean (SD)	3.25 (1.47)	3.13 (1.56)	3.10 (1.80)	3.16 (1.47)	
PLR, median (range)	150.00 (67.00–551.00)	141.00 (54.00–479.00)	141.00 (67.00–430.00)	142.00 (54.00–551.00)	0.627
PLR, mean (SD)	179.00 (95.69)	173.00 (91.15)	180.00 (95.01)	177.00 (87.90)	

ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; [1], *p*-value calculated using the Friedman test.

Table A2. The LMR, NLR, and PLR high and low patients in each measurement.

	Measurement						*p* [1]		
	First		Second		Third		Second vs. First	Third vs. First	Third vs. Second
	n (%)	CI_{95} for % [2]	*n* (%)	CI_{95} for % [2]	*n* (%)	CI_{95} for % [2]			
LMR									
LMR high (>2.6)	34 (56.7)	43.2–69.4	35 (58.3)	44.5–70.9	33 (55.0)	41.6–67.9	>0.999	0.791	0.607
LMR low (≤2.6)	26 (43.3)	30.6–56.8	25 (41.7)	29.1–55.1	27 (45.0)	32.1–58.4			
"True LMR-high/LMR-low", the patients who remained in the same group (LMR high or LMR low) after the second/third measurements	x	X	49 (81.7)	69.6–90.5	41 (68.3)	55.0–79.7	x	x	0.804

Table A2. *Cont.*

	Measurement						p [1]		
	First		Second		Third		Second vs. First	Third vs. First	Third vs. Second
	n (%)	CI_{95} for % [2]	*n* (%)	CI_{95} for % [2]	*n* (%)	CI_{95} for % [2]			
NLR									
NLR high (≥3.0)	23 (38.3)	26.1–51.8	27 (45.0)	32.1–58.4	21 (35.0)	23.1–48.4	0.455	0.754	0.146
NLR low (<3.0)	37 (61.7)	48.2–73.9	33 (55.0)	41.6–67.9	39 (65.0)	51.6–76.9			
"True NLR-high/NLR-low", the patients who remained in the same group (NLR high or NLR low) after the second/third measurements	x	X	44 (73.3)	60.3–83.9	41 (68.3)	55.0–79.7	x	x	0.146
PLR									
PLR high (≥150)	29 (48.3)	35.2–61.6	24 (40.0)	27.6–53.5	26 (43.3)	30.6–56.8	0.180	0.581	0.581
PLR low (<150)	31 (51.7)	38.4–64.8	36 (60.0)	46.5–72.4	34 (56.7)	43.2–69.4			
"True PLR-high/PLR-low", the patients who remained in the same group (PLR high or PLR low) after the second/third measurements	x	X	47 (78.3)	65.8–87.9	42 (70.0)	56.8–81.2	x	x	>0.999

LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; [1], comparison between measurements with the McNemar test; [2], 95% confidence interval (CI) for proportions based on a binomial exact calculation.

Table A3. Correlations between the LMR, NLR, and PLR and the CEA.

	CEA	
	r	***p*-Value**
LMR avg *	−0.01	0.964
NLR avg *	0.19	0.183
PLR avg *	0.22	0.108

LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CEA, carcinoembryonic antigen; *, average level from all three measurements; r, Spearman's correlation coefficient.

Table A4. Correlation between the CPS and the CD8+ lymphocytes and inflammatory infiltrates.

	CD8+		Inflammatory Infiltrates	
	r	***p*-Value**	**r**	***p*-Value**
CPS	0.56	0.002	0.51	0.005

CPS, combined positive score; r, Spearman's correlation coefficient.

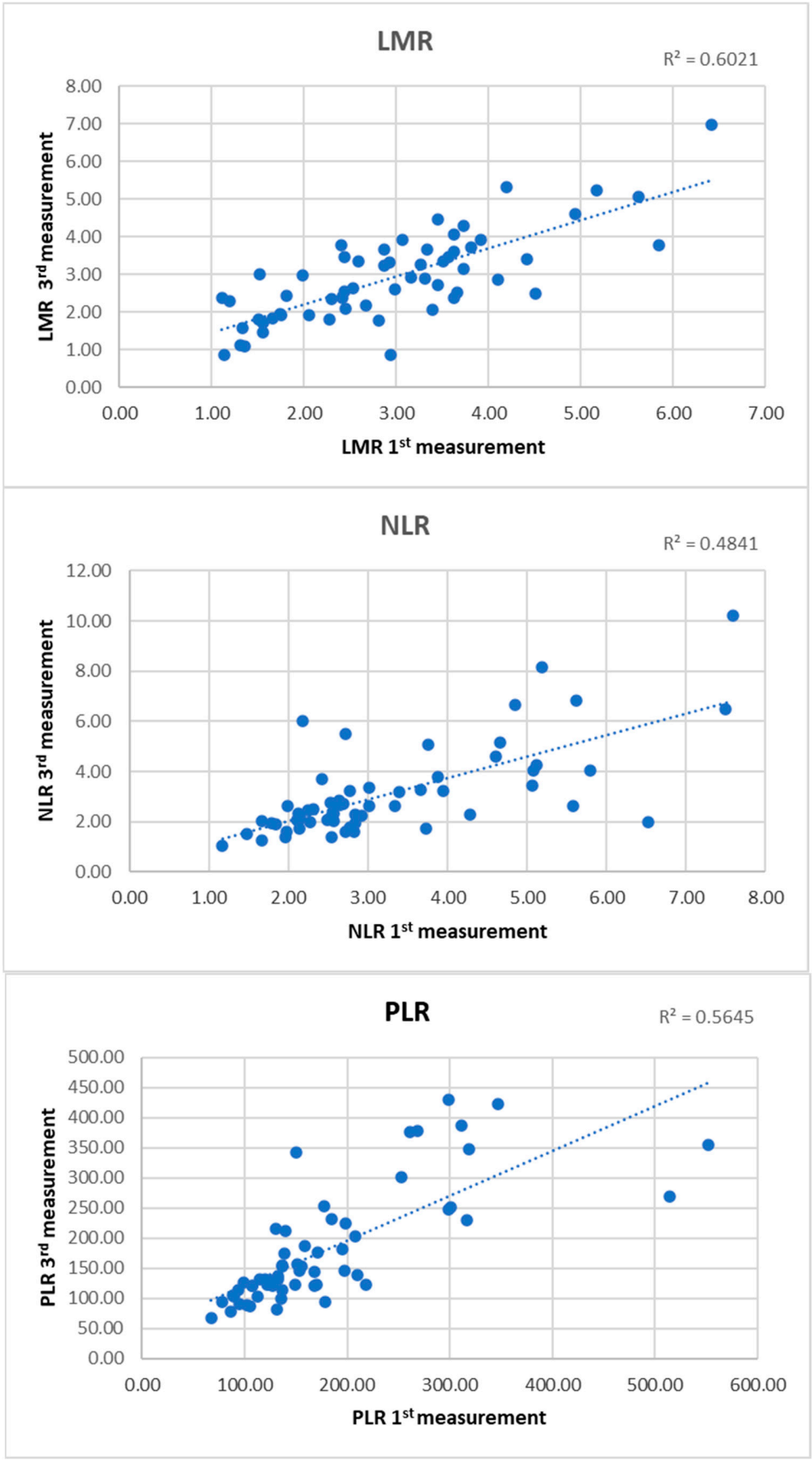

Figure A1. The coefficients of determination of the LMR, NLR, and PLR. LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.

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Publikacja 2.

"Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Extramural Vascular Invasion (EMVI) in Locally Advanced Rectal Cancer"

"Korelacja między współczynnikiem limfocytów do monocytów (LMR), neutrocytów do limfocytów (NLR) i trombocytów do limfocytów (PLR) a naciekaniem naczyń zlokalizowanych poza ścianą odbytnicy (EMVI) u pacjentów z miejscowo zaawansowanym rakiem odbytnicy"

Gawiński, C.; Hołdakowska, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Extramural Vascular Invasion (EMVI) in Locally Advanced Rectal Cancer. *Curr. Oncol.* **2023**, *30*, 545-558. https://doi.org/10.3390/curroncol30010043

Celem pracy była ocena zależności między markerami SIR: LMR, NLR i PLR oraz parametrami kliniczno-patologicznymi i biochemicznymi a naciekaniem naczyń zlokalizowanych poza ścianą odbytnicy (EMVI) u pacjentów z miejscowo zaawansowanym rakiem odbytnicy (LARC). Retrospektywnej analizie poddano 371 pacjentów z LARC leczonych w Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie − Państwowym Instytucie Badawczym w Warszawie między sierpniem 2016r. a grudniem 2021r. Kryteriami włączenia do badania było przeprowadzenie diagnostyki z zastosowaniem rezonansu magnetycznego (MRI) wysokiej rozdzielczości z oceną statusu EMVI oraz zakwalifikowanie przez zespół wielodyscyplinarny do przedoperacyjnej radio/radiochemioterapii. Kryteriami wyłączenia były współwystępujące nowotwory lite, stan po leczeniu immunosupresyjnym oraz obecność zaburzeń hematologicznych lub innych stanów chorobowych mogących istotnie wpłynąć na wykładniki stanu zapalnego. Stu osiemdziesięciu czterech pacjentów zostało włączonych do badania. U każdego pacjenta oceniano morfologię krwi obwodowej; mediana czasu między badaniem krwi a wykonaniem badania MRI wynosiła 8 dni. Ocena EMVI była przeprowadzana przez dwóch niezależnych radiologów z co najmniej 10-letnim doświadczeniem. Ocenie poddawano związki między LMR, NLR, PLR oraz parametrami kliniczno-patologicznymi i biochemicznymi a EMVI. Stwierdzono zależność między obecnością parametru EMVI w MRI, a klinicznym zaawansowaniem nowotworu (wielkością guza, zajęciem regionalnych węzłów chłonnych). Wykazano również istotnie statystycznie wyższy poziom antygenu karcynoembrionalnego (CEA) oraz neutrocytów i trombocytów u pacjentów EMVI-dodatnich w stosunku do pacjentów EMVI-ujemnych. Nie stwierdzono istotnych różnic w poziomach LMR, NLR i PLR w grupach pacjentów EMVI-dodatnich i EMVI-ujemnych. Nie stwierdzono korelacji między markerami SIR a parametrem EMVI.

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Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Extramural Vascular Invasion (EMVI) in Locally Advanced Rectal Cancer

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Abstract: Rectal cancer constitutes around one-third of all colorectal cancers. New markers are required to optimize the treatment. Extramural vascular invasion (EMVI) is a magnetic resonance imaging (MRI)-based negative prognostic marker. Lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) or platelet-to-lymphocyte ratio (PLR) are blood-based systemic inflammatory response markers with proven prognostic value in many cancers, including CRC. We hypothesized whether there is a relationship between LMR, NLR, PLR and the presence of EMVI on pre-treatment MRI in patients with locally advanced rectal cancer (LARC). We conducted a retrospective analysis of 371 patients with LARC treated in the Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland between August 2016 and December 2021. One hundred eighty-four patients were found eligible for the study. A correlation between the extension of the tumour, nodal status, clinical stage of the disease and the presence of EMVI was found ($p < 0.001$). The pre-treatment level of neutrophils, platelets and carcinoembryonic antigen (CEA) was significantly higher in the EMVI-positive population ($p = 0.041$, $p = 0.01$, $p = 0.027$, respectively). There were no significant differences regarding the level of LMR, NLR and PLR between the EMVI-positive and EMVI-negative population. LMR, NLR and PLR do not differentiate patients in terms of EMVI; neither of these parameters is a good predictor of the status of EMVI in LARC.

Keywords: lymphocyte-to-monocyte ratio (LMR); neutrophil-to-lymphocyte ratio (NLR); platelet-to-lymphocyte ratio (PLR); extramural vascular invasion (EMVI); rectal cancer

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1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer-related death worldwide [1] Rectal cancer, which constitutes around one-third of all CRCs, is distinct from colon cancer with different etiology, risk factors, pre-treatment staging and treatment strategies. The incidence of rectal cancer in the European Union is estimated to be as high as 125 000 per year and is expected to increase in the future [2]. Locally advanced rectal cancer (LARC) is usually defined as stage II or III disease (T3-T4, node-negative or node-positive irrespective of the extension of the tumor). Neoadjuvant radio/chemo-radiotherapy followed by surgery according to total mesorectal excision principles is deemed to be the standard of care. Treatment modalities differ substantially among countries and research centers. There is a growing need for novel markers in order to identify high-risk patients and optimize the treatment. Until recently, the Union for International Cancer Control (UICC) tumor node metastasis (TNM) staging system has been a backbone for treatment planning and main prognostic factor [3]. With the

development of magnetic resonance imaging (MRI) technology, high-resolution MRI has become the standard preoperative diagnostic tool in rectal cancer [4]. Extramural vascular invasion (EMVI) is defined as an invasion of malignant cells into blood vessels (usually veins). Detection of EMVI on pre-treatment MRI is associated with an increased risk of distant metastases and reduced disease-free survival. [5]. Blood-based systemic inflammatory response (SIR) markers are other emerging biomarkers with a well-established prognostic value in many cancers. Lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are among the most investigated ones. Low LMR, high NLR and high PLR are linked to unfavorable prognosis. This pattern has been consistently confirmed in various malignancies [6–8]. The prognostic value of SIR markers in colorectal cancer has been well documented in both locally advanced and metastatic stages [9–15]. We hypothesized whether there is a correlation between radiological and blood-based prognostic markers allowing for SIR markers to act as surrogates of the status of EMVI. In this study, we investigated the relationship between LMR, NLR, PLR and the presence of EMVI on pre-treatment MRI in LARC patients.

2. Materials and Methods

A retrospective analysis of a database of 371 patients with LARC treated in the Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland between August 2016 and December 2021 was performed. The inclusion criteria were as follows: (1) patients were diagnosed with primary locally advanced rectal cancer (T3-T4, N0-N2, M0) confirmed by histopathology. TNM stage was assessed according to the American Joint Committee on Cancer TNM staging standard, 8th edition; (2) pre-treatment staging with a high-resolution MRI scan of the pelvis and evaluation of EMVI status by an experienced radiologist was performed; (3) performance status of the patients was ECOG 0–2, patients were qualified to receive radio/chemo-radiotherapy by multidisciplinary team; (4) clinical records including demographic and laboratory data were available and complete. The exclusion criteria were: (1) presence of distant metastases after the diagnosis; (2) chemotherapy and/or radiotherapy applied prior to MRI; (3) presence of malignant tumors in other organs; (4) presence of hematologic malignancies and disorders that could substantially affect inflammatory markers; (5) prior immunosuppressive therapy. One hundred eighty-seven patients were excluded from the study due to unmet inclusion criteria or the presence of exclusion criteria. One hundred eighty-four were found eligible for the study as shown in Figure 1.

Blood examination of each patient has been analyzed and level of LMR, NLR and PLR has been calculated as presented in Table 1. The pre-treatment level of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) was collected when available (164/184 cases). The median time between blood examination and MRI was 8 days (range 0–43 days). The differential white blood cell count was analyzed using the Sysmex XN-550 hematology analyzer following the manufacturer protocol. All the patients received neoadjuvant radio/chemo-radiotherapy according to the multidisciplinary team decision based on the stage of the disease. The surgery was performed on 149 patients. Post-surgical pathological results were collected and analyzed.

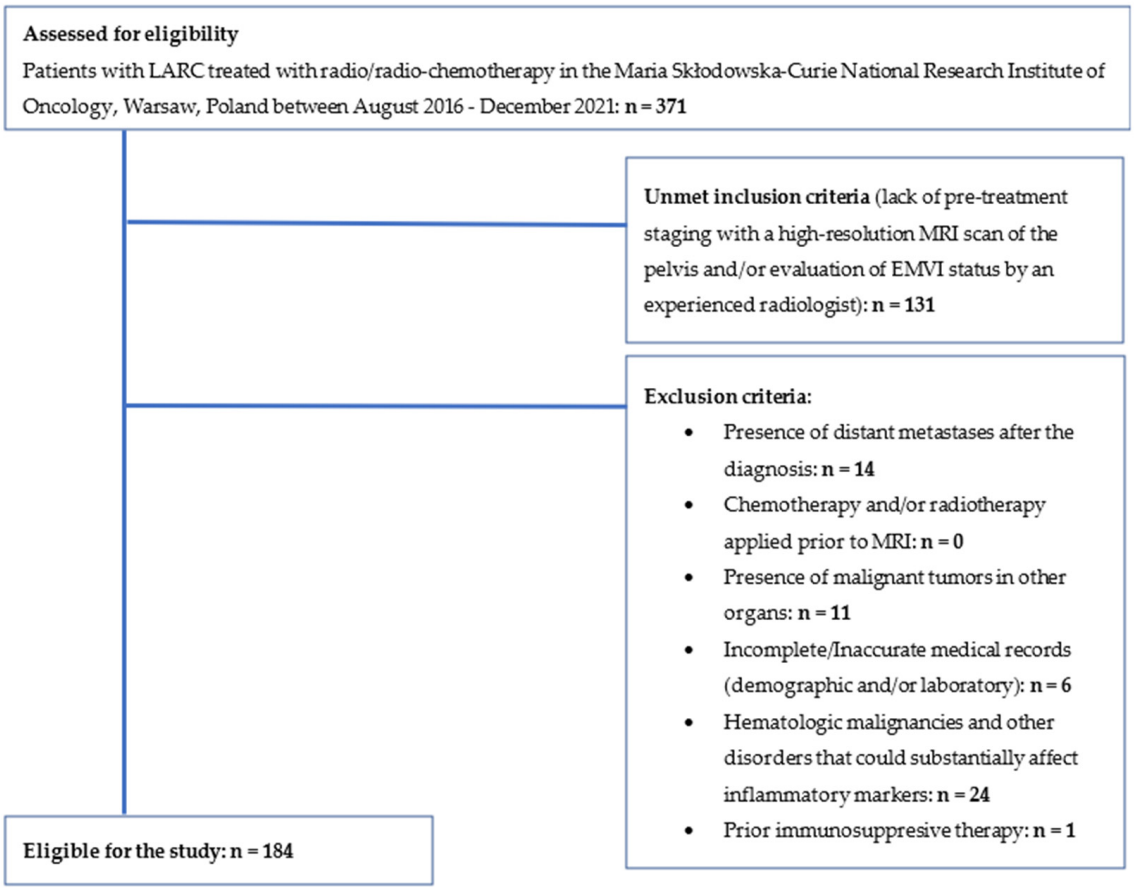

Figure 1. Eligibility for the study. LARC, locally advanced rectal cancer; MRI, magnetic resonance imaging; EMVI, extramural vascular invasion.

Table 1. Calculation of LMR, NLR and PLR.

Formulas:
LMR—absolute lymphocyte count (10^9/L)/absolute monocyte count (10^9/L)
NLR—absolute neutrophil count (10^9/L)/absolute lymphocyte count (10^9/L)
PLR—absolute platelet count (10^9/L)/absolute lymphocyte count (10^9/L)

LARC, locally advanced rectal cancer; MRI, magnetic resonance imaging; EMVI, extramural vascular invasion.

2.1. MRI Acquisition

The standard pelvic MRI was carried out using a 1.5 T-3T system, as routinely used for the clinical staging of patients with rectal cancer (according to the European Society of Gastrointestinal and Abdominal Radiology). The examination was performed with the use of a phased-array surface coil. Patients received intravenous spasmolytic; they did not receive a bowel preparation. According to the protocol two-dimensional (2D) FSE T2-weighted sequences without fat suppression, with a small field of view and a section thickness less than 3 mm (high-resolution protocol) and a diffusion-weighted sequence (including at least a high *b*-value of $\geq$800) have been performed. Transverse and coronal sequences were angulated perpendicular and parallel to the rectal tumor axis, respectively.

2.2. Assessment of Status of EMVI

EMVI, defined as the extension of tumor within the vessels of the mesorectum, was identified by the high-resolution MRI-based radiological features such as the vessel wall irregularity, focal enlargement, and/or signal intensity of the tumor within the vessel. To detect EMVI and minimize the risk of interobserver variability each MRI scan was reviewed independently by two radiologists with at least ten years of experience in pelvic MRI assessment. Equivocal cases were jointly discussed among experienced team of radiologists and consensus decision on the status of EMVI was made. EMVI was reported as positive/negative. Representative images of EMVI-negative and EMVI-positive rectal cancers are presented in Figures 2 and 3.

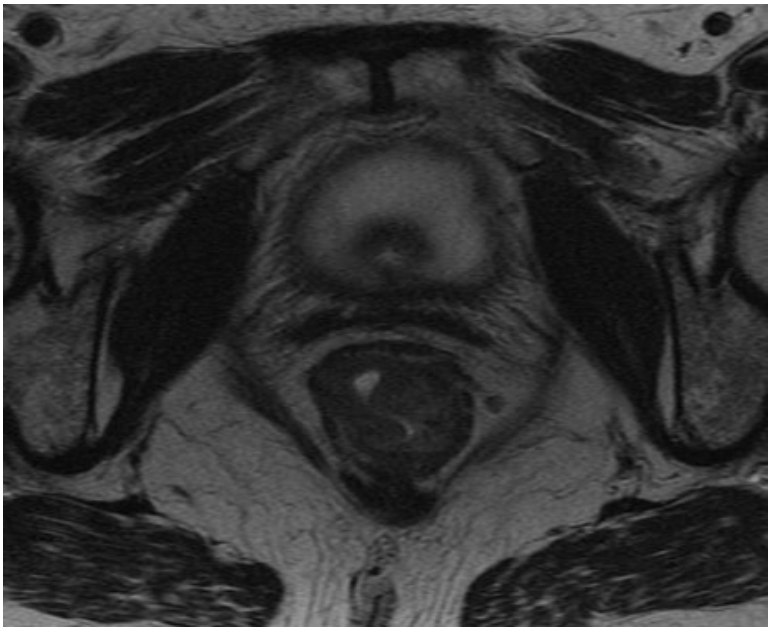

Figure 2. EMVI-negative rectal cancer.

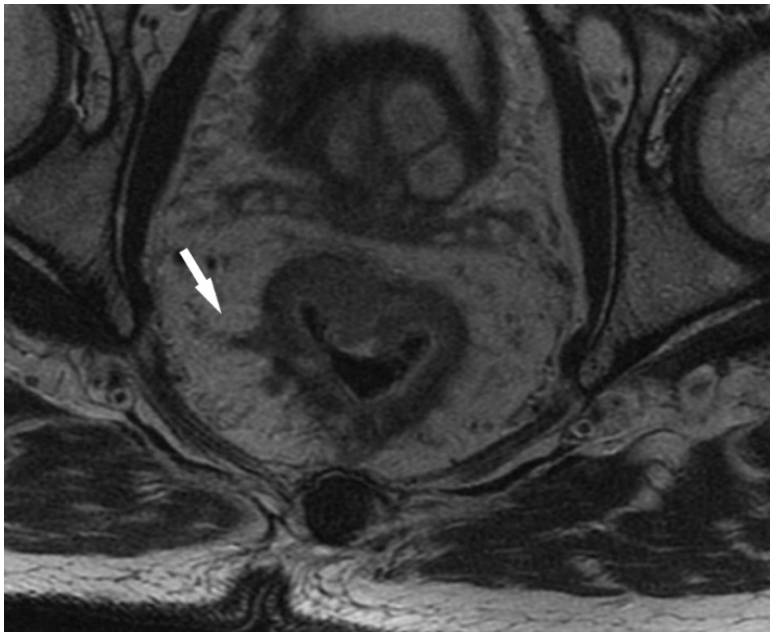

Figure 3. EMVI-positive rectal cancer. Axial T2-weighted MRI scan showing EMVI (white arrow).

2.3. Statistical Analysis

Analyses were conducted in statistical software R, version 4.2.2 (The R Foundation for Statistical Computing c/o Institute for Statistics and Mathematics Wirtschaftsuniversit¨at Wien Welthandelsplatz 1 1020 Vienna, Austria). The level of significance was equal to $\alpha = 0.05$. Dependencies between groups and other qualitative variables were analysed with chi-square test or Fisher's exact test. Quantitative variables were compared between groups using Mann–Whitney's test. Normality of distributions was analysed with Shapiro–Wilk's test. ROC (Receiver Operating Characteristic) analyses were performed to check whether the level of LMR, NLR and PLR differentiates patients in terms of EMVI—sensitivity, specificity and area under curve (AUC with 95% confidence intervals) were calculated, as well as optimal cut-off point for mentioned variables (based on Youden's criterium). Kendall's tau-b coefficient was used to assess the correlation between selected variables.

2.4. Ethical Considerations

The study conformed to the provisions of the Declaration of Helsinki and was approved by the ethics committee of Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw.

3. Results

The characteristics of patients is presented in Table 1. One hundred eleven males and seventy-three females were included in the study. The mean age was 66 years old (range, 36–87 years old). The distribution of cancer stages was as follows: stage II-IIIA—84 patients (47.2%); stage IIIB—65 (36.5%) patients and IIIC—29 (16.3%) patients. Seventy-eight patients (42.4%) were EMVI-positive and one hundred six patients (57.6%) were EMVI-negative. The mean and median values of lymphocytes, monocytes, neutrophils and thrombocytes counts are presented in Table 2. The median values of LMR, NLR and PLR were 2.87, 2.62 and 153.11, respectively. The median value of CEA was 3.88 ng/mL and CA 19-9 10.04 U/mL.

There was a greater proportion of patients with T2-T3 tumors in the EMVI-negative than in the EMVI-positive group (91% vs. 66%; $p < 0.001$). In the EMVI-negative group there was 53% of patients without nodal involvement and 10% of patients with nodal status N2. In the EMVI-positive group those proportions were, respectively, 26% and 35%—presenting a statistically significant dependency ($p < 0.001$). A greater proportion of patients with stage II-IIIA and lower proportion of patients with stage IIIC were found in the EMVI-negative group than in the EMVI-positive group (58% vs. 33% for stage II-IIIA and 5% vs. 32% for stage IIIC; $p < 0.001$). The level of neutrophils, platelets and CEA was significantly lower in the EMVI-negative group than in the EMVI-positive group ($p = 0.041$ for neutrocytes, $p < 0.001$ for platelets, and $p = 0.027$ for CEA), Table 3.

All the patients received neoadjuvant radiotherapy or chemo-radiotherapy based on multidisciplinary team decisions. A greater proportion of patients with pathological stage I or II and a smaller proportion of patients with pathological stage III could be observed among EMVI-negative patients than among EMVI-positive (27% vs. 13% for stage I, 36% vs. 25% for stage II, and 24% vs. 54% for stage III; $p = 0.002$). Among EMVI- negative group there was a greater proportion of patients with pN 0 than among EMVI-positive group (75% vs. 45%) and a smaller proportion of patients with pN 1 (17% vs. 37%) or pN 2 (8% vs. 18%); $p < 0.001$. No significant correlation was observed between pT and EMVI ($p = 0.078$).

Table 2. Characteristics of all patients.

Characteristics	Value
N	
EMVI +, n (%)	78 (42.4)
EMVI −, n (%)	106 (57.6)
Age (years), M ± SD, range (years)/ Me (Q1; Q3)	65.55 ± 10.83, 36–87/65.69 (58.97; 72.32)
Sex (female), n (%)	73 (39.9)
Tumor, n (%)	
T2-T3	146 (80.7)
T4	35 (19.3)
Lymph nodes, n (%)	
N0	68 (41.2)
N1	63 (38.2)
N2	34 (26.0)
Stage, n (%)	
II-III A	84 (47.2)
III B	65 (36.5)
III C	29 (16.3)
ALC (10^9/L), M ± SD	1.77 (1.38; 2.24)
AMC (10^9/L), Me (Q1; Q3)	0.60 (0.50; 0.76)
ANC (10^9/L), Me (Q1; Q3)	4.77 (3.77; 5.99)
Platelets (10^9/L), Me (Q1; Q3)	274.50 (230.75; 337.25)
LMR, Me (Q1; Q3)	2.87 (2.25; 3.81)
NLR, Me (Q1; Q3)	2.62 (2.09; 3.42)
PLR, Me (Q1; Q3)	153.11 (117.98; 204.10)
CEA (ng/mL), Me (Q1; Q3)	3.88 (2.33; 9.06)
CA19-9 (U/mL), Me (Q1; Q3)	10.04 (4.64; 17.73)

EMVI, extramural vascular invasion; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; M, mean; SD, standard deviation; Q, quartile; Me, median.

Table 3. Comparison of characteristics between EMVI groups.

Characteristics	EMVI −	EMVI +	*p*
Age (years), Me (Q1; Q3)	65.90 (58.75; 74.00)	64.90 (59.48; 71.76)	0.667
Sex (female), n (%)	43 (41.0)	30 (38.5)	0.851[2]
Tumor, n (%)			
T2-T3	96 (91.4)	50 (65.8)	**<0.001**[2]
T4	9 (8.6)	26 (34.2)	
Lymph nodes, n (%)			
N0	49 (52.7)	19 (26.4)	**<0.001**[2]
N1	35 (37.6)	28 (38.9)	
N2	9 (9.7)	25 (34.7)	
Stage, n (%)			
II-III A	59 (57.8)	25 (32.9)	**<0.001**[2]
III B	38 (37.3)	27 (35.5)	
III C	5 (4.9)	24 (31.6)	
ALC (10^9/L), Me (Q1; Q3)	1.70 (1.33; 2.24)	1.90 (1.45; 2.26)	0.217
AMC (10^9/L), Me (Q1; Q3)	0.58 (0.46; 0.80)	0.63 (0.53; 0.75)	0.181
ANC (10^9/L), Me (Q1; Q3)	4.49 (3.62; 5.81)	5.10 (4.07; 6.16)	**0.041**
Platelets (10^9/L), Me (Q1; Q3)	253.50 (217.00; 313.25)	303.00 (249.50; 361.75)	**0.001**
LMR, Me (Q1; Q3)	2.90 (2.30; 3.83)	2.86 (2.20; 3.79)	0.885
NLR, Me (Q1; Q3)	2.54 (2.05; 3.31)	2.82 (2.18; 3.43)	0.189
PLR, Me (Q1; Q3)	150.70 (115.97; 200.00)	167.19 (119.95; 218.05)	0.293
LMR above median, n (%)	53 (50.0)	38 (48.7)	0.982[2]
NLR above median, n (%)	49 (46.2)	43 (55.1)	0.296[2]
PLR above median, n (%)	49 (46.2)	43 (55.1)	0.296[2]
CEA, Me (Q1; Q3)	3.62 (2.24; 6.97)	5.63 (3.07; 9.68)	**0.027**
CA19-9, Me (Q1; Q3)	9.87 (5.36; 15.76)	11.71 (2.77; 18.29)	0.741

Bold font indicates statistical significance. EMVI, extramural vascular invasion; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; Q, quartile; Me, median. Differences in the level of quantitative variables between groups were analysed with Mann–Whitney's U test, dependencies between qualitative variables and groups were analysed with chi-square test[2] (with Yate's correction for continuity for 2 × 2 tables).

No significant correlation between pCR and the status of EMVI was detected (8.2% in EMVI-positive and 13% in EMVI-negative group; $p = 0.350$), Table 4.

Table 4. Correlations between EMVI and pathological staging.

Characteristics	EMVI −	EMVI +	*p*
Stage, n (%)			
pCR	12 (13.0)	5 (8.2)	0.350
I	25 (27.2)	8 (13.1)	
II	33 (35.9)	15 (24.6)	**0.002**
III	22 (23.9)	33 (54.1)	
pT, n (%)			
0	10 (11.2)	4 (6.7)	
1 and 2	30 (33.7)	12 (20.0)	0.078
3 and 4	49 (55.1)	44 (73.3)	
pN, n (%)			
0	67 (75.3)	27 (45.0)	
1	15 (16.9)	22 (36.7)	**<0.001**
2	7 (7.9)	11 (18.3)	

Bold font indicates statistical significance. EMVI, extramural vascular invasion; pCR, pathological complete response. Dependencies between qualitative variables and groups were analysed with chi-square test.

Patients were assigned to groups based on the change between the MRI-based clinical TNM-staging and the post-surgical pathological TNM-staging depending on the pre-treatment status of EMVI. No significant dependency was detected between EMVI and the change of the stage, T or N status ($p > 0.050$ for all variables), Supplementary Table S1.

The optimal cut-off point for level of LMR was 2.62 (sensitivity = 0.63; 1-specificity = 0.57). The AUC (area under curve) equalled 0.49 (95% CI = 0.41; 0.58), which meant that LMR level was not a good predictor of the status of EMVI, Figure 4.

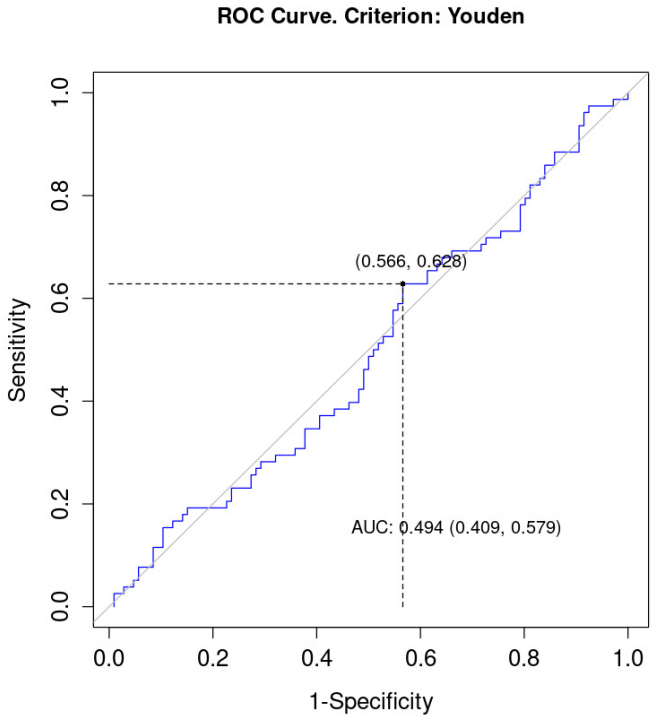

Figure 4. ROC (Receiver Operating Characteristic) curve for LMR as a discriminator of EMVI. AUC, area under curve.

The AUC for NLR was 0.56 (95% CI = 0.47; 0.64) which poorly differentiated patients in terms of the status of EMVI. Both sensitivity and 1-specificity were low (0.54 and 0.39, respectively). The optimal cut-off point for NLR was 2.78, Figure 5.

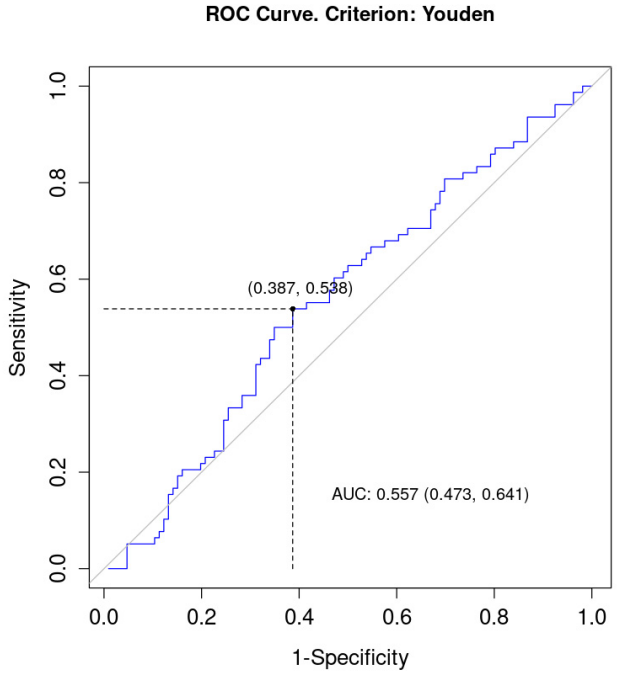

Figure 5. ROC (Receiver Operating Characteristic) curve for NLR as a discriminator of EMVI. AUC, area under curve.

The AUC for PLR was also higher than 0.50 and equaled 0.55 (95% CI = 0.46; 0.63), which meant that PLR was a weak discriminator of the status of EMVI. The sensitivity for this variable was 0.54, and 1-specificity equaled 0.40. The optimal cut-off point in this model was 162.71, Figure 6.

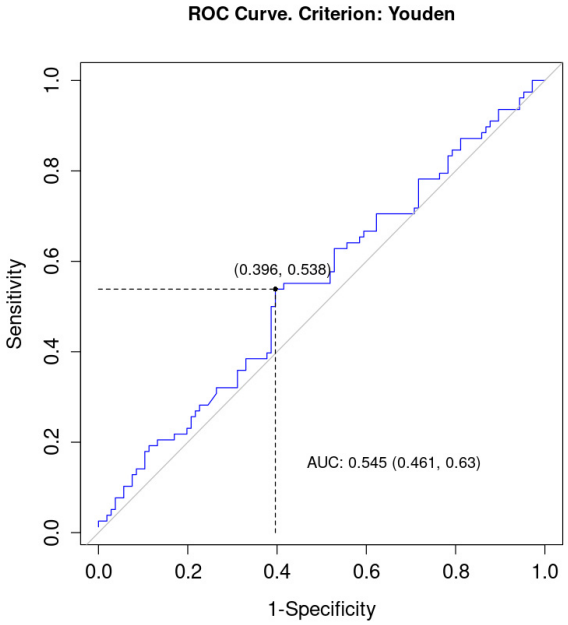

Figure 6. ROC (Receiver Operating Characteristic) curve for PLR as a discriminator of EMVI. AUC, area under curve.

In all the analysed groups of patients according to the level of LMR, NLR and PLR there was a significant correlation between the status of EMVI and the stage of the disease.

In EMVI-positive population there were more stages IIIC and fewer stages II-IIIA than in EMVI-negative population independently from the median value of LMR, NLR and PLR ($p < 0.050$ for all analyses), Table 5.

Table 5. Dependency between stage of the disease and EMVI (broken down into groups by the level of LMR, NLR and PLR).

Stage, n (%)	EMVI −	EMVI +	*p*
	LMR ≤ median		
II–III A	29 (58.0)	15 (38.5)	
III B	19 (38.0)	11 (28.2)	**0.002**
III C	2 (4.0)	13 (33.3)	
	LMR > median		
II–III A	30 (57.7)	10 (27.0)	
III B	19 (36.5)	16 (43.2)	**0.002**
III C	3 (5.8)	11 (29.7)	
	NLR ≤ median		
II–III A	30 (55.6)	12 (34.3)	
III B	21 (38.9)	13 (37.1)	**0.008**
III C	3 (5.6)	10 (28.6)	
	NLR > median		
II–III A	29 (60.4)	13 (31.7)	
III B	17 (35.4)	14 (34.1)	**0.001**
III C	2 (4.2)	14 (34.1)	
	PLR ≤ median		
II–III A	31 (56.4)	10 (29.4)	
III B	21 (38.2)	16 (47.1)	**0.010**
III C	3 (5.5)	8 (23.5)	
	PLR > median		
II–III A	28 (59.6)	15 (35.7)	
III B	17 (36.2)	11 (26.2)	**<0.001**
III C	2 (4.3)	16 (38.1)	

Bold font indicates statistical significance. EMVI, extramural vascular invasion; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio. Data presented as n (% of EMVI−/EMVI + group). Dependencies were analysed with chi-square test.

No significant dependency was observed between pre-treatment level of CEA, CA19-9 and: LMR, NLR, PLR level ($p > 0.050$ for all correlation analyses), Table 6.

Table 6. Correlation analysis between CEA, CA19-9 and LMR, NLR, PLR.

Variables	CEA		CA19-9	
	tau-b	*p*	tau-b	*p*
LMR	0.03	0.634	−0.06	0.306
NLR	<0.01	0.964	0.04	0.526
PLR	0.04	0.506	−0.07	0.226

LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; tau-b, Kendall's correlation coefficient; *p*, *p* value for correlation analysis.

4. Discussion

The goal of the study was to investigate the relation between the pre-treatment MRI-based status of EMVI and SIR markers: LMR, NLR and PLR. As far as we know, our study is the first to evaluate directly such correlations. A statistically significant association between the status of EMVI and the extension of the tumor, nodal status and the clinical stage of the disease has been observed in our study. Correlation between EMVI and the stage of the disease was observed among patients with both high and low levels of LMR, NLR and PLR. A correlation between pre-treatment levels of neutrophils, thrombocytes, carcinoembryonic antigen and the status of EMVI was found ($p = 0.041$; $p = 0.001$ and $p = 0.027$, respectively). In an analysis of correlation between pre-treatment status of EMVI and pathological TNM staging there was a significant correlation between EMVI and the pathological stage ($p = 0.002$) and the pN status ($p < 0.001$). There was no significant difference in the frequency of complete pathological response in EMVI-positive and EMVI-negative patients ($p = 0.350$). The status of EMVI on pre-treatment MRI had no statistically significant impact on the change of the stage of the disease between MRI-based and post-surgical pathological TNM staging.

In accordance with our results, the relation between MRI-detected EMVI-positive rectal cancers and positive nodal status and advanced T-stage has been demonstrated in several studies [16–18]. In spite of significant correlation between level of neutrophils, platelets and EMVI, no such phenomenon between EMVI and LMR, NLR or PLR was detected. The available data on relations between SIR markers and EMVI in the literature is scarce. In a study by Li et al., no statistically significant association between EMVI and NLR and PLR was found in locally advanced rectal cancer patients [19]. Pine et al. investigated the relationship between NLR and the tumor characteristics and local lymphocytic response to tumor. NLR was associated with more advanced, aggressive tumor biology, lymph node metastases and EMVI ($p = 0.07$). However, the status of EMVI in the study was assessed pathologically (pEMVI), the investigated population was not confined to rectal cancer patients but included colon cancers as well and the cut-off value of NLR was relatively high (5.0 compared to 2.86 in our study) [20]. The data on correlation between EMVI and tumor markers (CEA, CA19-9) are contradictory [21,22]. No reliable information on the relation between thrombocytosis and neutrophilia and the status of EMVI has been found in the literature. EMVI has been proved to be a reliable marker of worse survival and disease recurrence [23,24]. Traditionally, EMVI was detected on pathological analysis of the resected specimens (pEMVI); however, it was demonstrated to lead to substantial under-reporting. In historical studies the incidence of pEMVI ranged from 9% to 90% as a result of an inconsistency in pathological definition and often inability to distinguish lymphatic invasion from venous invasion. In recent years, it has been proved that EMVI may be identified by high-resolution MRI at least as or more accurately compared to routine pathological analysis [25,26].

In rectal cancers EMVI has become a standard of care in pre-operative radiological assessment. In most reports it is estimated to be present in around 35% of these tumors varying from 20% to over 50% [27,28]. The role of (neo)adjuvant chemotherapy in LARC is uncertain. There is no high-quality evidence for its benefit, but it is commonly used based on data extrapolated from trials of colon cancers.

However, there is a significant variability in practice as to which treatment modalities should be offered for this group of patients. EMVI is increasingly used as one of the main factors helping to identify patients who require more intense peri-operative treatment. Chand et al. demonstrated that patients with EMVI-positive stage II tumors have a similarly increased risk of developing metastases as EMVI-negative stage III patients [29]. Meta-analysis by Siddiqui et al. revealed a significantly increased risk of both synchronous and metachronous metastases in patients with MRI-based EMVI. [28]. EMVI's role is not limited to rectum—it's been shown to be a strong predictor of worse oncological outcomes in stage II–III colon cancer patients; studies show promising data concerning its possible prognostic value in esophageal and gastric cancers (via computed tomography EMVI) as well [30–32].

LMR, NLR and PLR are novel blood-based biomarkers with strong prognostic value in various malignancies. Each of these parameters take advantage of the specific role and impact of lymphocytes, monocytes, neutrophils and platelets on immunological system. Lymphocytes through cytotoxic activity and production of anti-tumor cytokines play a key role in suppressing cancer's proliferation and spread [33]. On the contrary, high count of monocytes, neutrocytes and platelets contribute to cancer initiation, angiogenesis, tumor progression and metastatic activity [34–38]. These properties have led to the introduction of lymphocyte-to-monocyte, neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios with strong prognostic value. In LARC low LMR and high NLR and PLR are unfavorable prognostic factors and therefore potential biomarkers which may be used to identify high-risk patients requiring more intense treatment [10,39,40].

EMVI has been well established with SIR markers as an emerging status as prognostic factors in rectal cancer. We hypothesized that combining these parameters may enhance their prognostic value and allow for more accurate pre-treatment assessment of patients (e.g., as a part of risk-scoring models) in the future. The correlation between EMVI and SIR markers could allow to utilize cheap and easy blood-based markers as surrogates of the status of EMVI in situations when MRI is contraindicated or where the accessibility of pre-treatment MRI is still low. The main limitation of our study is a high interobserver variability in the assessment of the status of EMVI [41]. Despite the independent evaluation of each MRI scan by at least two experienced radiologists, its impact on the results is possible.

Despite the fact that no correlation between LMR, NLR, PLR and the status of EMVI was found; interesting dependencies with the clinicopathological features and pre-treatment level of neutrophils, platelets and CEA were demonstrated.

We believe our research is novel and the subject of correlations between radiological and hematological markers in rectal cancer is almost absent in the literature. We hope this study will attract more attention to the topic and encourage further investigation.

5. Conclusions

The presence of EMVI on pre-treatment MRI is one of the most important negative prognostic factors in rectal cancer. LMR, NLR and PLR are emerging blood-based markers with powerful prognostic value in CRC. We conducted novel research investigating the relationship between these parameters in locally advanced rectal cancer. LMR, NLR and PLR do not differentiate patients in terms of EMVI; none of these parameters seem to be a good predictor of the status of EMVI. However, the status of EMVI has been well correlated with many clinicopathological features and hematological markers. Further investigations on the possibility of utilizing these correlations in clinical practice are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/curroncol30010043/s1, Table S1: Change between MRI-based clinical TNM-staging and post-surgical pathological TNM-staging depending on the pre-treatment status of EMVI.

Author Contributions: Conceptualization, C.G. and L.W.; methodology, C.G. and L.W.; software, C.G.; validation, L.W.; formal analysis, C.G.; investigation, C.G.; resources, C.G.; data curation, C.G.; writing—original draft preparation, C.G. and A.H; writing—review and editing, C.G., A.H. and L.W.; visualization, C.G. and A.H.; supervision, L.W. and A.H.; project administration, C.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study conformed to the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw (KB-55/2020).

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to medical data privacy issues.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Publikacja 3.

"Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Tumor-Infiltrating Lymphocytes (TILs) in Left-Sided Colorectal Cancer Patients"

"Korelacja między współczynnikiem limfocytów do monocytów (LMR), neutrocytów do limfocytów (NLR) i trombocytów do limfocytów (PLR) a limfocytami naciekającymi guz nowotworowy (TILs) u pacjentów z lewostronnym rakiem jelita grubego i odbytnicy"

Gawiński, C.; Michalski, W.; Mróz, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Tumor-Infiltrating Lymphocytes (TILs) in Left-Sided Colorectal Cancer Patients. *Biology* **2022**, *11*, 385. https://doi.org/10.3390/biology11030385

Celem badania była ocena zależności między obwodowymi a lokalnymi markerami reakcji zapalnej związanej z chorobą nowotworową oraz analiza ich wartości prognostycznej. Oceniano parametry z krwi obwodowej: LMR, NLR i PLR oraz zaburzenia immunologiczne w mikrośrodowisku guza. Retrospektywnej analizie poddano 87 pacjentów z miejscowo zaawansowanym lewostronnym rakiem jelita grubego i górnej odbytnicy zakwalifikowanych decyzją zespołu wielodyscyplinarnego do radykalnego leczenia operacyjnego. Pacjenci poddawani byli leczeniu chirurgicznemu w Narodowym Instytucie Onkologii im. Marii Skłodowskiej-Curie − Państwowym Instytucie Badawczym w Warszawie między styczniem 2014r. a grudniem 2015r. Do badania włączono pacjentów z rakiem dystalnej esicy, złącza esiczo-odbytniczego lub górnej odbytnicy. Kryteriami wyłączenia z badania była obecność współwystępujących nowotworów, wcześniejsze stosowanie chemio- i/lub radioterapii, obecność zaburzeń hematologicznych lub innych stanów chorobowych mogących istotnie wpłynąć na wykładniki stanu zapalnego lub stosowanie leczenia immunosupresyjnego w przeszłości. Analizowano wyniki morfologii krwi obwodowej pobranej przed zabiegiem operacyjnym. Pooperacyjny materiał histopatologiczny z usuniętym guzem nowotworowym był oceniany pod kątem gęstości limfocytów CD3+ i CD8+ w centrum guza i w jego inwazyjnym marginesie. W badaniu wzięło udział 50 pacjentów. Stwierdzono znamienną statystycznie różnicę w poziomie limfocytów CD3+ w centrum guza między pacjentami z niskimi i wysokimi poziomami NLR. W 6-letniej obserwacji wykazano istotnie statystyczny związek między wysokimi wartościami LMR oraz niskimi wartościami NLR a dłuższym OS. Nie stwierdzono prognostycznej wartości gęstości limfocytów CD3+ lub CD8+ w tkance guza.

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Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Tumor-Infiltrating Lymphocytes (TILs) in Left-Sided Colorectal Cancer Patients

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Simple Summary: Colorectal cancer (CRC) is one of the most common cancers worldwide. Novel markers have been investigated in order to better predict the course of disease and adjust the treatment. Markers associated with cancer-related inflammation (CRI), both in the bloodstream and the tumor tissue, have been in the spotlight for years. In this study, we investigate whether blood-based markers: lymphocyte-to-monocyte ratio, neutrophil-to-lymphocyte ratio or platelet-to-lymphocyte ratio, correlate with tissue-based markers, such as tumor-infiltrating lymphocytes. We retrospectively analyzed 87 patients with locally advanced left-sided CRC treated with radical surgery. Fifty patients were found suitable for the study. We compared the results of their blood tests from the time of the surgical intervention and the density of lymphocytes in the resected tumors. We found no correlation between local and peripheral markers of CRI. Further prospective studies are needed to confirm the results.

Abstract: Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide. Novel markers are required in order to select high-risk patients and better adjust the treatment. Both peripheral and local markers of cancer-related inflammation (CRI) such as lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) or platelet-to-lymphocyte ratio (PLR) and tumor-infiltrating lymphocytes (TILs) have been thoroughly investigated in recent years and deemed to be highly prognostic. We hypothesized that there is an association between local and peripheral CRI indices and that blood-based biomarkers may serve as a surrogate of TILs. We retrospectively analyzed 87 patients with locally advanced left-sided CRC treated with radical-intent surgery in the Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw, Poland, between January 2014 and December 2015. Fifty patients were found eligible for the study. The patients were divided in terms of pre-treatment values of systemic inflammatory response (SIR) markers into LMR/NLR/PLR-high and low groups. We evaluated the resected specimens by immunohistochemistry in order to assess the densities of CD3+ and CD8+ lymphocytes in the center of the tumor and in the invasive margin. We found that the level of CD3+ lymphocytes in the center of the tumor was statistically significantly higher in patients with low pre-treatment NLR ($p = 0.044$); however, no correlation between any of the SIR markers and CD3+ or CD8+ TILs was observed. Five-year overall survival (OS) was longer in patients with high LMR ($p < 0.001$), low NLR ($p = 0.001$) and low PLR ($p = 0.095$). No correlation between the density of TILs and OS was demonstrated. In conclusion, based on our study, peripheral blood-based markers and CD3+ and CD8+ TILs are not interrelated.

Keywords: lymphocyte-to-monocyte ratio (LMR); neutrophil-to-lymphocyte ratio (NLR); platelet-to-lymphocyte ratio (PLR); tumor-infiltrating lymphocyte (TIL); colorectal cancer (CRC)

1. Introduction

Colorectal cancer is the third most common cancer and the second most common cause of cancer-related death worldwide [1]. Despite advances in surgical procedures and adjuvant chemotherapy, approximately 20% of patients still experience relapse following curative treatment [2]. The Union for International Cancer Control (UICC) tumor node metastasis (TNM) staging system is the most reliable indicator of patient prognosis and is widely used among practitioners to determine the most appropriate therapy [3]. However, prognosis may differ substantially even within the same TNM stage. Therefore, new reliable markers are required to improve predictions on the course of disease and lead to a more adjusted treatment. Cancer-related inflammation (CRI) indices, both in the peripheral blood and in the tumor microenvironment, may be suitable for this role. Peripheral systemic inflammatory response (SIR) markers such as LMR, NLR or PLR have potent prognostic value in many malignancies [4,5,6]. The "Immunoscore"—a value based on the density of CD3+ and CD8+ TILs in the tumor center (CT) and the invasive margin (IM)—has been shown to be highly prognostic in colon cancer. Some reports have suggested the superior role of the Immunoscore in predicting survival compared to the TNM staging system [7]. In this study, we tried to evaluate the correlation between local (TILs) and peripheral (LMR, NLR and PLR) CRI biomarkers in CRC patients.

2. Materials and Methods

We performed a retrospective analysis of a database of 87 patients treated with radical-intent surgery in the Department of Gastrointestinal Cancers, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland, between January 2014 and December 2015. The inclusion criteria were as follows: (1) histologically confirmed cancer of distal sigmoid, rectosigmoid or upper rectum (> 10 cm from the anal verge by colonoscopy); (2) no evidence of tumor invading the adjacent organs or distant metastasis; (3) no neoadjuvant treatment applied; and (4) presence of formalin-fixed tissues from the surgical excision of the tumor. The exclusion criteria were: (1) metastatic disease; (2) neoadjuvant chemotherapy and/or radiotherapy; (3) malignant disease of other organs; (4) presence of hematologic malignancies and disorders that could substantially affect inflammatory markers; (5) prior immunosuppressive therapy; and (6) incomplete/inaccurate medical records. Thirty-seven patients were excluded from the study due to the presence of exclusion criteria (10 patients) or the fact that histological specimens were found inadequate for appropriate pathomorphological assessment (27 patients) as shown in Box. 1.

Box 1. Eligibility for the study.

87 patients with left-sided colorectal cancer (distal sigmoid, rectosigmoid, upper rectum) treated with radical-intent surgery in the Department of Gastrointestinal Cancers, Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland between January 2014 - December 2015.

Exclusion criteria:

- Metastatic disease found postoperatively-3 patients
- Malignant disease of other organs-1 patient
- Incomplete/Inaccurate medical records-5 patients
- Hematologic malignancies and disorders that could substantially affect inflammatory markers-1 patient.

Histological specimens found inadequate for appropriate pathomorphological assessment–27 patients.

50 patients eligible for the study

We analyzed a routine blood examination before surgery of each patient and calculated their LMR, NLR and PLR by dividing an absolute count of lymphocytes by an absolute count of monocytes, an absolute count of neutrophils by an absolute count of lymphocytes and an absolute count of thrombocytes by an absolute count of lymphocytes in peripheral blood, respectively, as presented in box 2. The median time between a blood test and the surgery was 3 days (range from 1 to 11 days). The differential white blood cell count was analyzed using the Sysmex XN-550 hematology analyzer following the manufacturer's protocol.

Box 2. Calculation of LMR, NLR, PLR.

Formulas:

LMR—absolute lymphocyte count (109/l) / absolute monocyte count (10 9/l)

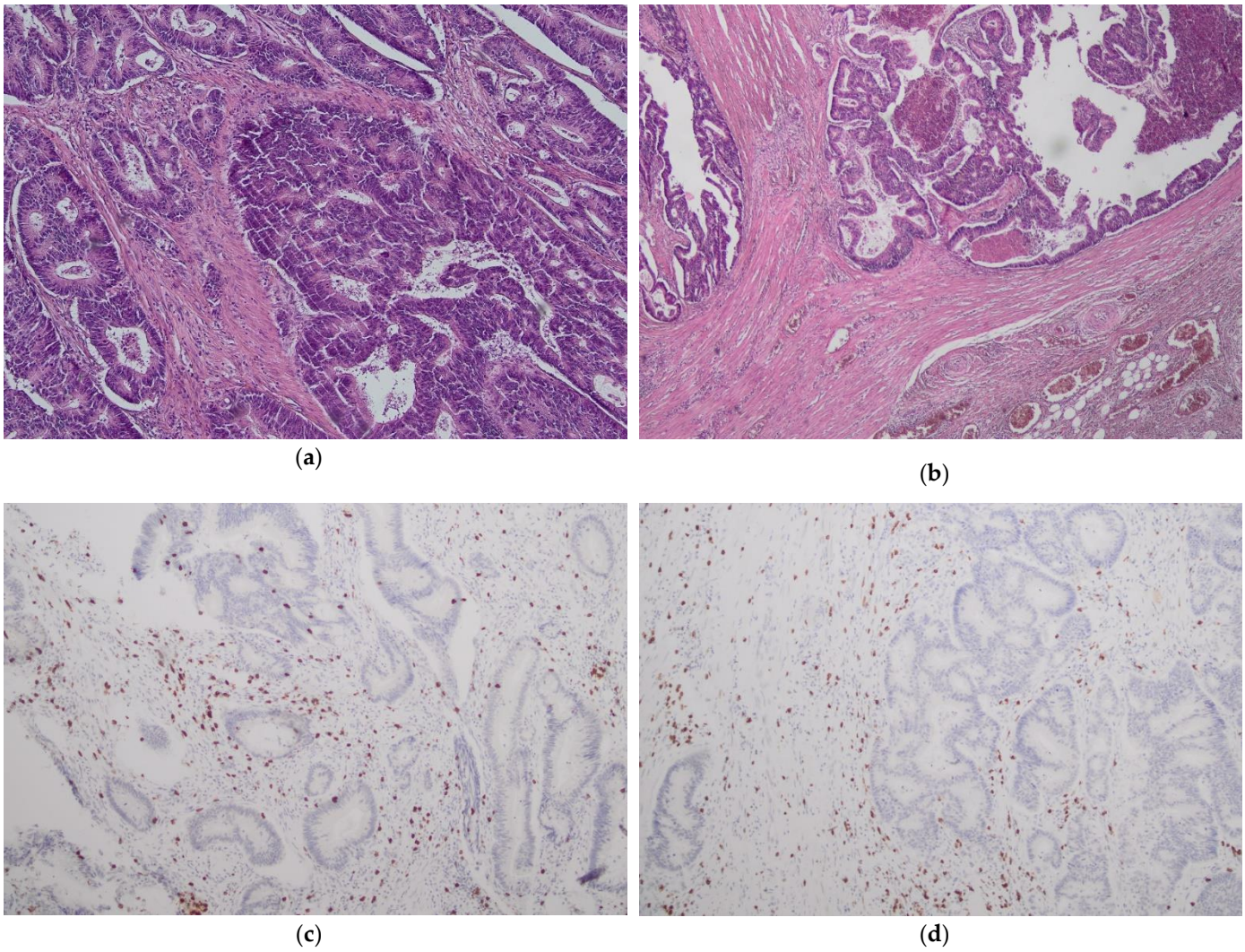
NLR—absolute neutrophil count (109/l) / absolute lymphocyte count (10 9/l)

PLR—absolute platelet count (109/l) / absolute lymphocyte count (10 9/l)

The patients were divided in terms of pre-treatment values of SIR markers. The cut-off values were predetermined based on available data in the literature and our previous studies [4, 6, 8, 9]. For LMR, the cut-off value was 2.6, for NLR 3.0 and for PLR 150.

2.1. Immunohistochemistry

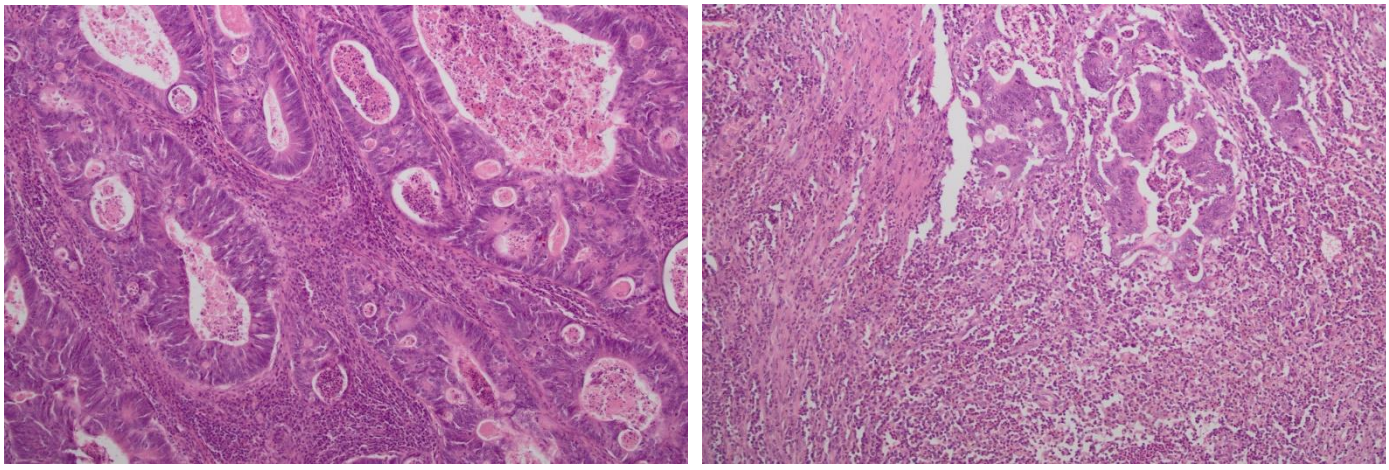
The presence of tumor-infiltrating immune cells in the tumor center and the invasive margin was evaluated by immunohistochemistry using the antibodies for CD3 and CD8 antigens. For immunohistochemical staining, we used primary monoclonal antibodies against CD3 (DAKO, Glostrup, Denmark, Cat. No M7254) and CD8 (DAKO, Denmark, Cat. No IR623) with a DAKO EnVision FLEX detection system (DAKO, Denmark, Cat. No K8002). Paraffin sections (4 μm on silanized slides) were deparaffinized and rehydrated. Antigen epitopes were retrieved by the high temperature method (high pH in PT link). Sections were incubated with primary antibody (20 min), and EnVision FLEX+ target retrieval solution was used. Finally, a color reaction was achieved by incubation with EnVision FLEX DAB chromogen (10 min at room temperature) and hematoxylin counterstain was used for nuclei visualization. Semi-quantitative analysis by an experienced pathologist and a quantitative automated analysis of the specimens was performed. In a semi-quantitative assessment, a four-digit scale (0: 0–10% of the area of scarce and mild staining, 1: 11–50% of the area of moderate or intensive staining, 2: 50–75% of the area of intermediate or intensive staining and 3: > 75% area of intermediate or intensive staining) of density of lymphocytes was used in separate measurements for tumor center and invasive margin while in a quantitative assessment an exact number of lymphocytes per 1 mm^2 of specimen was calculated (using the CellSens Software version 1.16 by Olympus). The highest lymphocyte density regions were selected for histological and immunohistochemical assessment. The representative images of low and high lymphocyte infiltrates in CT and IM are presented in Figures 1 and 2.



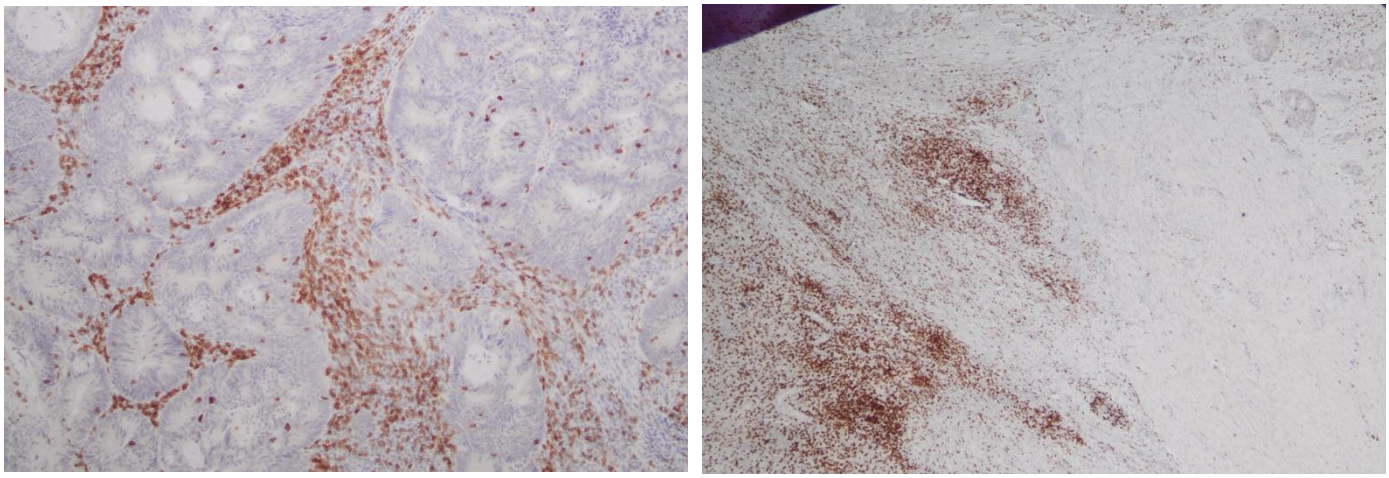
(**a**) (**b**)

(**c**) (**d**)

Figure 1. No/minimal lymphocyte infiltrates in tumor center (CT) (**a,c**) and tumor margin (IM) (**b,d**). H&E staining x40 (**a**); H&E staining ×100 (**b**) ; CD3 staining ×100 (**c,d**).



(**a**) (**b**)



(**c**) (**d**)

Figure 2. Intense lymphocyte infiltrates in tumor center (CT) (**a**,**c**) and tumor margin (IM) (**b**,**d**). H&E staining x40 (**a**); H&E staining ×100 (**b**) ; CD3 staining ×100 (**c**,**d**).

2.2. Statistical Analysis

The Shapiro–Wilk test was used to test the normality of the data distribution. The correlation between preoperative LMR, NLR, PLR and the density of CD3+ and CD8+ lymphocytes were analyzed using the Spearman's test. Comparison of parameters between patients according to the stage of the disease was carried out with the Kruskal–Wallis test, while the comparison of TILs according to the pre-treatment value of SIR markers with the Mann–Whitney U test. The Kaplan–Meier survival estimator was calculated, and logrank test was used to compare overall survival for SIR markers and TILs. All statistical analyses were performed using the IBM SPSS Statistics ver. 23 software package. A p-value < 0.05 was considered statistically significant.

2.3. Ethical Considerations

The study conformed to the provisions of the Declaration of Helsinki and was approved by the ethics committee of Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw.

3. Results

Fifty patients were found to be eligible for the study. As presented in Table 1, included in the study were 26 males and 24 females; median age at the initial surgery was 67 years old (range, 44–88 years). The median value of pre-treatment LMR was 3.16 (range 0.95–7.2). The medians of NLR and PLR were 2.34 (0.7–14.54) and 140 (58–358), respectively.

Resected specimens were pathologically classified according to the UICC TNM classification of malignant tumors, ver. 7. The distribution of cancer stages was as follows: stage I-11/50 (22%); stage II–18/50 (36%); stage III-21/50 (44%) patients. Tumor grade was mostly G2 (32 cases), followed by G1 (10) and G3 (5). In three cases, the grade remained indeterminate.

The densities of CD3+ and CD8+ lymphocytes were evaluated in the CT and IM of the resected tumors. In three cases, CD3+ and CD8+ lymphocytes were detected only in the invasive margin and not in the center of the specimen. The mean density of CD3+ lymphocytes per 1 mm^2 was 1699 (range 704–3900) in CT and 1929 (368–4959) in IM. The densities of CD8+ lymphocytes in CT and IM were 877 (66–3918) and 1255 (175–2511), respectively.

Table 1. Characteristics.

Characteristic	All Patients (*n* = 50)
Age (years), median (range)	67 (44–88)
Sex, n (%)	
Male	26 (52.0)
Female	24 (48.0)
Tumor, n (%)	
T1-T2	13 (26.0)
T3-T4	37 (74.0)
Lymph nodes, n (%)	
N0	29 (58.0)
N1-N2	21 (42.0)
Grade, n (%)	
G1	10 (20.0)
G2	32 (64.0)
G3	5 (10.0)
Gx	3 (6.0)
Stage, n (%)	
I	11 (22.0)
II	18 (36.0)
III	21 (42.0)
ALC (10^9/l), median (range)	1.94 (0.69–3.95)
AMC (10^9/l), median (range)	0.57 (0.30–1.14)
ANC (10^9/l), median (range)	4.10 (2.01–10.03)
Platelets (10^9/l), median (range)	246 (153–430)
LMR, median (range)	3.16 (0.95–7.20)
NLR, median (range)	2.34 (0.70–14.54)
PLR, median (range)	140 (58–358)
CD3 CT/mm^2, mean (range)	1699 (704–3900)
CD3 IM/mm^2, mean (range)	1929 (368–4959)
CD8 CT/mm^2, mean (range)	877 (66–3918)
CD8 IM/mm^2, mean (range)	1255 (175–2511)

ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CT, tumor center; IM, invasive margin.

There was a statistically significant correlation between the density of both CD3+ and CD8+ cells in CT and IM ($p \leq 0.005$), Table 2.

Table 2. Correlation between CD3+ and CD8+ TILs in CT and IM.

TILs	CD3 CT		CD3 IM		CD8 CT		CD8 IM	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***
CD3 CT	x	x	0.52	< 0.001	0.52	< 0.001	0.40	0.005
CD3 IM	0.52	< 0.001	x	x	0.59	< 0.001	0.69	< 0.001
CD8 CT	0.52	< 0.001	0.59	< 0.001	x	x	0.59	< 0.001
CD8 IM	0.40	0.005	0.69	< 0.001	0.59	< 0.001	x	x

TILs, tumor-infiltrating lymphocytes; CT, tumor center; IM, invasive margin; r, correlation coefficient calculated with Spearman's rho test.

Median value of SIR markers and TILs have been evaluated according to the stage of the disease. No statistically significant differences in the level of parameters between the stages of the disease were found, as presented in Table 3 ($p > 0.05$ in all cases).

Table 3. Comparison of SIR markers and TILs according to the stage of the disease.

SIR markers/TILs	Stage I	Stage II	Stage III	*p*
LMR	2.88 (1.13–7.20)	3.42 (2.30–6.00)	3.00 (0.95–6.23)	0.501
NLR	3.66 (0.70–13.09)	2.15 (1.20–4.64)	2.17 (0.97–14.54)	0.579
PLR	135.00 (58.00–268.00)	140.00 (61.00–187.00)	140.00 (66.00–358.00)	0.910
CD3 CT/mm²	1602 (704–2745)	1771 (705–3336)	1687 (716–3900)	0.829
CD3 IM/mm²	1795 (636–2818)	1913 (368–3920)	2006 (900–4959)	0.965
CD8 CT/mm²	939 (66–3918)	973 (432–3445)	734 (161–2664)	0.226
CD8 IM/mm²	1042 (236–1827)	1302 (232–2511)	1325 (175–2432)	0.464

SIR, systemic inflammatory response; TILs, tumor-infiltrating lymphocytes; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CT, tumor center; IM, invasive margin. Data presented as median (range). Groups compared with Kruskal–Wallis test.

We found no correlation between pre-treatment LMR, NLR, PLR and the density of CD3+ and CD8+ TILs, Table 4.

Table 4. Correlation between LMR, NLR, PLR and CD3+ and CD8+ TILs in CT and IM.

TSIR markers	CD3 CT		CD3 IM		CD8 CT		CD8 IM	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***
LMR	0.08	0.575	0.03	0.857	0.19	0.195	0.03	0.854
NLR	0.05	0.720	0.08	0.606	0.10	0.496	0.03	0.843
PLR	0.07	0.645	0.12	0.424	0.03	0.831	0.09	0.518

SIR, systemic inflammatory response; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CT, tumor center; IM, invasive margin; r, correlation coefficient calculated with Spearman's rho test.

The Semi-Quantitative Evaluation

There were no significant differences in the average level of semi-quantitative evaluation of CD3+ and CD8+ lymphocytes between groups of pre-treatment LMR above/below 2.6, NLR above/below 3.0 and PLR above/below 150, as presented in Table 5.

Table 5. Comparison of TILs according to pre-treatment values of SIR markers.

TILs	LMR			NLR			PLR		
	LMR ≤ 2.6	**LMR > 2.6**	***p***	**NLR ≥ 3.0**	**NLR < 3.0**	***p***	**PLR ≥ 150**	**PLR < 150**	***p***
s-q CD3 CT	1 (0–3)	2 (0–3)	0.287	2 (0-2)	1 (0–3)	0.909	2 (0–3)	1 (0–3)	0.553
q CD3 CT/mm²	1201.79 (703.57–3 900.00)	1544.64 (705.36–3 335.71)	0.213	1233.93 (703.57–2 950.00)	1767.86 (705.36–3 900.00)	0.044	1289.29 (703.57–3 107.14)	1517.86 (705.36–3 900.00)	0.868
s-q CD3 IM	1 (0-3)	2 (0–3)	0.076	1 (0–3)	2 (0–3)	0.204	1 (0–3)	2 (0–3)	0.760
q CD3 IM/mm²	1560.71 (1 112.50–4 958.93)	1783.93 (367.86–3 919.64)	0.688	1541.07 (367.86–2 248.21)	1971.43 (635.71–4 958.93)	0.061	1682.14 (367.86–3 919.64)	1783.93 (635.71–4 958.93)	0.862
s-q CD8 CT	0 (0–2)	1 (0-3)	0.199	1 (0–2)	1 (0–3)	0.807	1 (0–3)	1 (0–2)	0.579
q CD8 CT/mm²	566.07 (160.71–2 664.29)	787.50 (66.07–3 917.86)	0.196	727.68 (160.71–1 266.07)	721.43 (66.07–3 917.86)	0.879	789.29 (171.43–3 917.86)	682.14 (66.07–3 444.64)	0.682
s-q CD8 IM	1 (0-3)	1 (0–3)	0.317	1 (0–2)	1 (0–3)	0.448	1 (0–3)	1 (0–2)	0.533
q CD8 IM/mm²	1196.43 (553.57–2 432.14)	1119.64 (175.00–2 510.71)	0.930	1075.89 (564.29–1 826.79)	1208.93 (175.00–2 510.71)	0.790	1196.43 (523.21–2 510.71)	1158.04 (175.00–2 330.36)	0.440

TILs, tumor-infiltrating lymphocytes; s-q, semi-quantitative; q, quantitative; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-monocyte ratio; PLR, platelet-to-lymphocyte ratio; CT, tumor center; IM, invasive margin. Data presented as median (range). Groups comparison with Mann–Whitney U test.

4. The Quantitative Evaluation

In the quantitative assessment, level of CD3+ in CT was significantly higher in NLR < 3.0 than NLR ≥ 3.0 patients, median of 1767.86 (range: 705.36–3900.00) vs. 1233.93 (703.57–2950.00), respectively ($p = 0.044$). No differences in the average level of quantitative evaluation of CD3+ and CD8+ lymphocytes between the groups of pre-treatment LMR above/below 2.6 and PLR above/below 150 were found, as presented in Table 5.

The Analysis of Overall-Survival (OS)

Patients participating and eligible for the study treated in Maria Sklodowska-Curie National Research Institute of Oncology between March 2014 and September 2015 were followed-up until December 21, 2020. The median follow-up time was over 6 years.

During this time, 36% (18/50) of patients died, and 64% (32/50) remained alive. Patients with pre-treatment LMR > 2.6 had a statistically significant longer OS than patients with LMR ≤ 2.6 ($p < 0.001$). Similarly, patients with baseline NLR > 3 had a statistically shorter OS than those with NLR ≥ 3 ($p = 0.001$). We observed a tendency towards better OS in patients with PLR ≤ 150 compared to PLR > 150; however, the result was statistically insignificant ($p = 0.095$) (Figures 3–5).

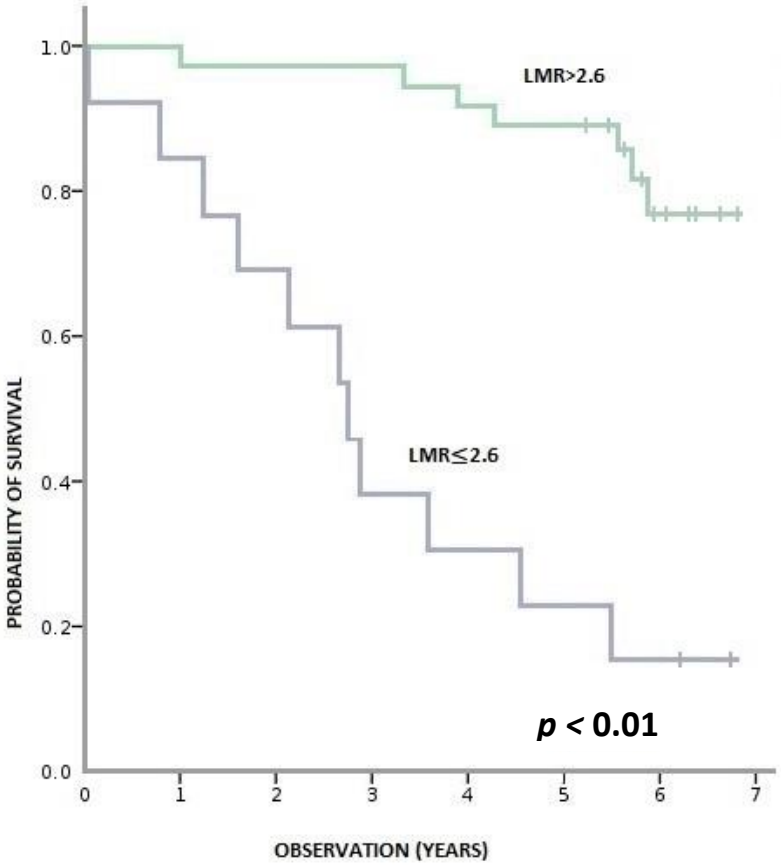


Figure 3. Overall survival according to pre-treatment LMR (lymphocyte-to-monocyte ratio).

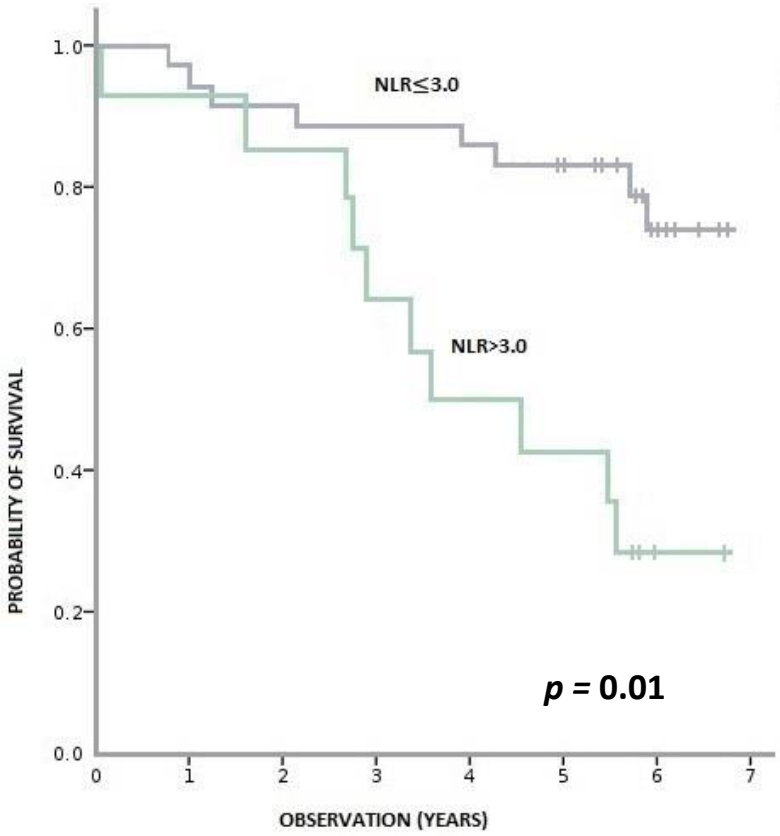


Figure 4. Overall survival according to pre-treatment NLR (neutrophil-to-monocyte ratio).

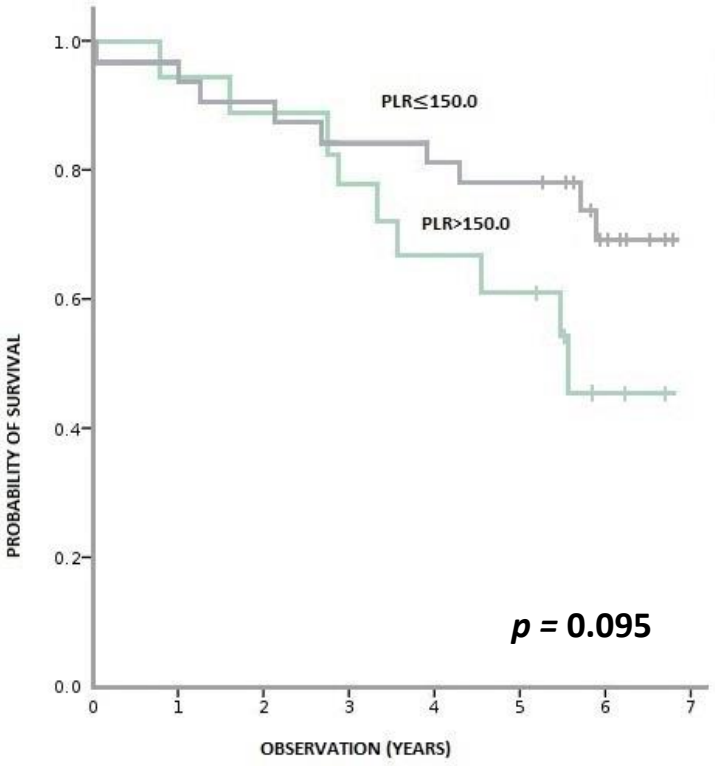


Figure 5. Overall survival according to pre-treatment PLR (platelet-to-lymphocyte ratio).

We found no correlation between levels of CD3+ and CD8+ lymphocytes in the cancer tissue and OS (Appendix A, Figures A1–A4).

5. Discussion

Our hypothesis was that there is a correlation between peripheral and local CRI markers and that SIR markers such as LMR, NLR and PLR may act as peripheral blood-based surrogates of TILs. The goal was to analyze the relationship between these parameters in left-sided CRC. Few studies have investigated this subject, and available data is scarce. The definition of TILs varies between studies as often different types of lymphocytes (CD3+, CD4+, CD5+, CD8+, CD45RO+, etc.) are taken into account and scored accordingly to different gradings. Moreover, there are no established cut-off values for SIR markers or TILs.

We observed a higher number of immune cells in IM than in CT and a positive correlation between the densities of CD3+ and CD8+ cells in both tumor regions, which is in accordance with available data from other reports.

Mean values of CD3+ and CD8+ lymphocytes in our study were high compared to some analyses; however, even higher mean densities of lymphocytes, especially in early stages of the disease, and higher intrapatient variability of the density of lymphocytes have been reported [10–13]. In most studies, only semi-quantitative evaluations of TIL densities were conducted, making it difficult to perform conclusive comparisons in this regard. Our analysis showed that patients with a pre-treatment value of NLR < 3.0 had a statistically significantly higher level of CD3+ lymphocytes in the center of the resected tumor compared to the NLR-high group. However, based on Spearman's rho test, no correlation between pre-treatment values of LMR, NLR and PLR and the density of CD3+ or CD8+ TILs was observed. The results of other studies concerning the relation between LMR, NLR, PLR and density of TILs are conflicting. Kwan Ho Lee et al. assessed a relationship between TILs and hematologic parameters in breast cancer. A statistically significant correlation between lymphocyte and monocyte count, LMR and CD8+ TILs has been demonstrated [14]. In another study, high TILs (CD3+, CD15+ and CD68+) were significantly correlated with low NLR and high LMR in locally advanced triple-negative breast cancer [15]. High preoperative NLR was associated with low TILs in hepatocellular carcinoma [16]. In patients who underwent curative surgery for gastric cancer, CD3+ and CD8+ immune cells densities were not associated with pre-treatment NLR [17]. A negative correlation between NLR and CD3+ was detected in patients with non-small cell lung cancer [18]. In CRC, a relationship between TILs and SIR markers has been observed mainly indirectly throughout common prognostic properties [19, 20]. The results of more direct correlations are scarce and unclear. In a study by Guo et al., high values of LMR were associated with a high intratumoral number of CD3+ T cells in CT. However, no correlations between either LMR and CD3+ T-cells in IM or between CD3+ T-cells and NLR or PLR were found. No correlations between CD8+ lymphocytes and LMR, NLR or PLR were detected either [21]. In a study evaluating rectal cancer patients, there was no correlation between baseline NLR and CD8+ lymphocytes; CD3+ T-cells, LMR or PLR were not evaluated [22].

Our study focused on investigating the association between inflammatory markers in circulating blood and tumor tissue rather than on prognostic outcomes; we did, however, perform an analysis of the association between peripheral and local CRI markers and OS. Despite the fact that there was no correlation between peripheral and local CRI markers and disease stage, strong prognostic values of LMR, NLR and, to a lesser extent, PLR were confirmed among our patients in accordance with most studies [23–25]. A correlation between the density of TILs and OS was, nonetheless, not observed. This phenomenon is not consistent with the majority of other results [26, 27]. However, a number of studies also failed to show the expected correlation—entirely or, at least, in some of the analyzed cohorts [28–31]. It is speculated that the lack of correlation between density of

TILs and survival or other prognostic factors (e.g., stage of disease) may be due to environmental variables, such as the microbiome or tumor inflammatory status [32, 33].

Possibly, the differences in tumor biology and immune microenvironment may affect the severity of the impairment of T-cells and the sensitivity of tumor cells to their cytotoxic functions affecting the immunological response [30].

In order to thoroughly understand the role and potential of peripheral and local inflammatory indices, it is crucial to comprehend the impact of each of their components on CRI. The relationship between inflammation and cancer is well-proven. This phenomenon was first discovered in the 19th century by observing inflammatory cells in resected tumor tissues and associated sites of chronic inflammation with carcinogenesis [34]. CRI affects many aspects of malignancy. It involves not only a reaction of the host's immune system against the tumor, but also inflammatory chemokines and cytokines released by tumor-associated leukocytes and cancer cells contributing to tumor growth, invasion and metastatic activity [35]. Lymphocyte count reflects the responsiveness of the immune system of the host. Lymphocytes inhibit cancer proliferation and spread [36]. Lymphopenia is often observed in advanced cancer and may result in a weak and insufficient immunological response. Studies have linked it with unfavorable prognosis in oncological patients [37]. By contrast, monocytes, neutrocytes and platelets play a vital role in tumor progression [38–40]. A correlation between monocytosis and a poor prognosis has been reported in many cancers [41]. Neutrophils, as the most common subset of leukocytes, have a substantial impact on CRI. They have been shown to play an important role in the initiation and progression of cancer [42]. Like monocytes, a high count of neutrophils has been associated with unfavorable outcomes in many malignancies [43]. Similarly, an elevated level of thrombocytes has been linked to a poor prognosis. Tumor cells, through cytokines and interleukins, stimulate megakaryocytes to induce the production and activation of thrombocytes. In turn, thrombocytes release angiogenic and growth factors, such as vascular endothelial growth factor and platelet-derived growth factor, substantially contributing to angiogenesis and tumor growth [44, 45]. Peripheral blood-based biomarkers, such as LMR, NLR and PLR, take advantage of the combined prognostic value of the mentioned blood components. CRI affects tumor microenvironments to a large extent. Tumor-infiltrating immune cells (TIICs) are composed mainly of immunological cells, such as tumor-associated macrophages, dendritic cells, mast cells and lymphocytes. TILs—white-blood cells originating from the bloodstream, which migrated towards the tumor site—are the most investigated subpopulation of TIICs. TILs are involved in the recognition and elimination of tumor cells and play an important role in boosting anti-tumor immunity [46]. Their prognostic and predictive role is well-established in breast cancer, especially the TNBC subtype, where a high level of TILs is correlated with better OS, disease-free survival and higher pathological complete response rate following neoadjuvant therapy [47, 48]. TILs are also correlated with an improved prognosis in several other cancers, such as lung, ovarian and pancreatic [49–51]. In CRC, an Immunoscore—a classification evaluating two lymphocyte populations (CD3+/CD45RO+, CD3+/CD8+ or CD8+/CD45RO+)—both in the CT and IM was developed. According to some reports, the Immunoscore is a superior predictor of OS and DFS compared to the AJCC/UICC TNM classification system in CRC [7].

In designing our study, we based it on the Immunoscore—we evaluated the quantitative and semi-quantitative density of populations of CD3+ and CD8+ lymphocytes in CT and IM. We focused on cancers of left-sided colon (distal sigmoid and rectosigmoid) and the upper part of the rectum, excluding middle and low rectal cancers, as it was essential for us to avoid any presurgical treatment (radio or chemotherapy) that could influence inflammation indices both in peripheral blood and in the tumor tissue. Low and middle rectal cancers, unlike those in the upper rectum and colon, are often treated with neoadjuvant radio/radio-chemotherapy. It is important to note that we decided to study tissues from the surgical excision of the tumor, not the presurgical biopsies, as standard biopsies often do not include an invasive margin area, which is the primary site of interaction

between malignant and immune cells [10]. Surgical specimens, however, allow an ample evaluation of tissue sections, especially with regard to invasive margins. The main limitation of our study was the small number of patients. The study was also retrospective, and we cannot exclude some patients that had non-cancer-related inflammation that could have affected the outcomes. Therefore, the results need to be repeated in bigger cohorts in prospective trials. However, it is worth noting that we conducted a novel study on the subject that is surprisingly absent in the literature. We hope our study will attract more attention to the topic and encourage further investigation.

6. Conclusions

Both blood-based SIR markers and tumor-infiltrating immune cells have been thoroughly investigated in recent years as prognostic factors in many cancers. They reflect a peripheral and local aspect of the immunological reaction within the CRI. To our knowledge, our study is the first to evaluate a direct correlation between LMR, NLR, PLR and TILs in left-sided colorectal cancer. We found that a level of CD3+ lymphocytes in the center of the tumor was significantly higher in patients with low pre-treatment NLR; however, no correlation between any of the pre-treatment blood-based markers and CD3+ or CD8+ lymphocytes in the resected tumor was demonstrated. Further investigations on a larger scale are crucial in order to better understand this relation.

Author Contributions: Conceptualization, C.G. and L.W.; methodology, C.G., L.W., A.M.; software, W.M.; validation, L.W., A.M. and W.M.; formal analysis, C.G; investigation, C.G.; resources, C.G.; data curation, C.G.; writing—original draft preparation, C.G.; writing—review and editing, C.G. and L.W.; visualization, C.G.; supervision, L.W.; project administration, C.G. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study conformed to the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of Maria Skłodowska-Curie National Research Institute of Oncology in Warsaw (KB-078-13/2017; 27 December 2017).

Informed Consent Statement: Patient consent was waived due to the fact that the study was retrospective, and according to the Ethics Committee, all patients who have been treated in Maria Skłodowska-Curie National Research Institute of Oncology and have undergone surgery had given their general consent to use their histopathological specimen and medical records in clinical studies.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to medical data privacy issues.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

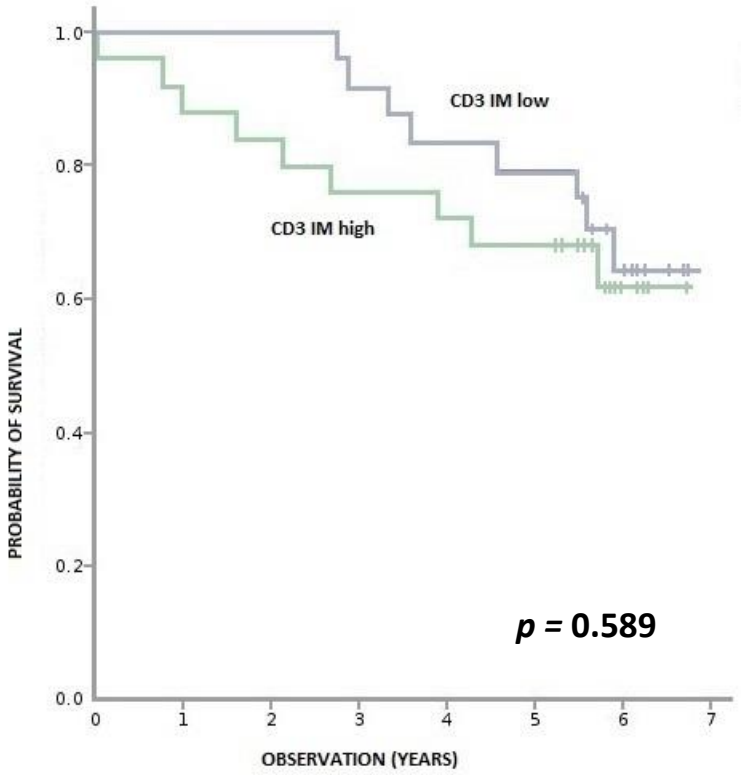

Figure A1. Overall survival according to density of CD3+ lymphocytes in IM (invasive margin). CD3+ IM high-≥ 970 per 0.56 mm^2, CD3+ IM low - < 970 per 0.56 mm^2.

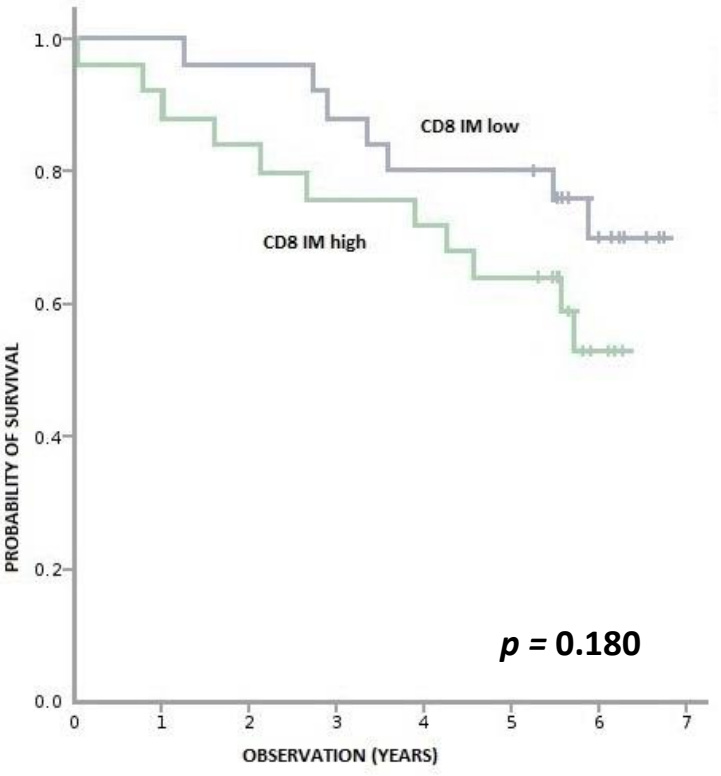

Figure A2. Overall survival according to density of CD8+ lymphocytes in IM (invasive margin). CD8+ IM high - ≥ 670 per 0.56 mm^2, CD8+ IM low - <670 per 0.56 mm^2.

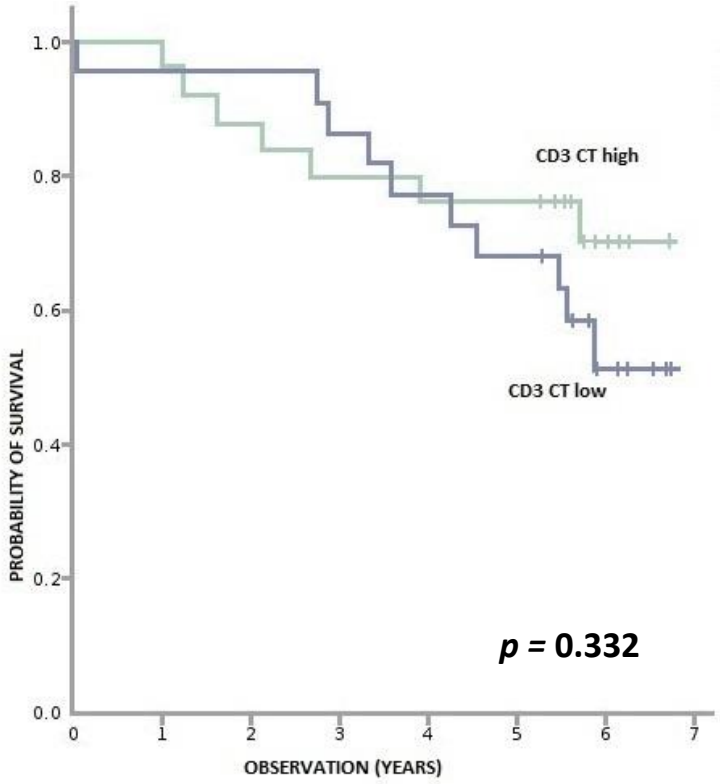

Figure A3. Overall survival according to density of CD3+ lymphocytes in CT (tumor center). CD3+ CT high - ≥ 840 per 0.56 $mm^{2,}$ CD3+ CT low - <840 per 0.56 mm^{2}.

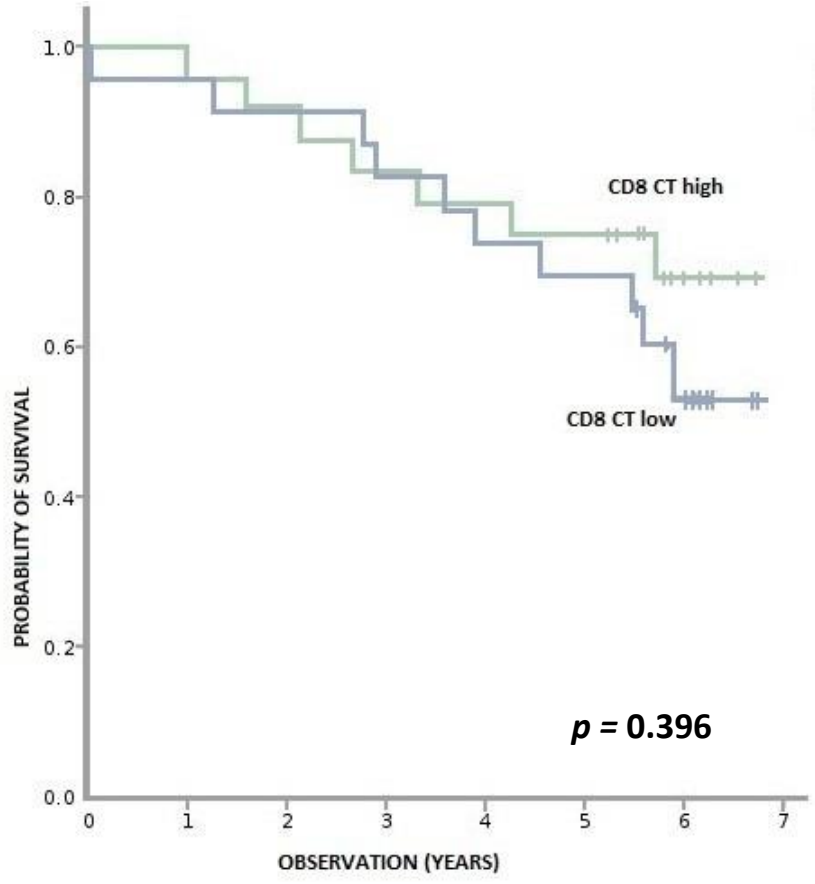

Figure A4. Overall survival according to density of CD8+ lymphocytes in CT (tumor center). CD8+ CT high - ≥ 404 per 0.56 $mm^{2,}$ CD8+ CT low - < 404 per 0.56 mm^{2}.

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Podsumowanie

Reakcja zapalna związana z chorobą nowotworową (CRI) jest zjawiskiem, które wpływa na zaburzenia immunologiczne zarówno w mikrośrodowisku guza jak i obwodowo w krwioobiegu. LMR, NLR i PLR są szeroko badanymi w onkologii wykładnikami CRI o potwierdzonej wartości prognostycznej w wielu nowotworach. Łatwość ich oceny i niski koszt badania czynią je potencjalnie dobrymi markerami, które mogą mieć zastosowanie w praktyce klinicznej. Celem zbioru badań wchodzących w skład niniejszej pracy doktorskiej była ocena ich powtarzalności, związku z parametrami kliniczno-patologicznymi, biochemicznymi i radiologicznymi oraz ocena wartości prognostycznej w polskiej populacji.

W badaniach stwierdzono istotne zależności pomiędzy obwodowymi i lokalnymi markerami CRI. Wykazano korelację między LMR a naciekiem zapalnym w mikrośrodowisku guza. Stwierdzono również zależność między niskimi wartościami NLR a wysoką gęstością limfocytów CD3+. Ponadto zaobserwowano relację pomiędzy wysokimi wartościami LMR a ekspresją PD-L1 na komórkach nowotworowych, limfocytach i makrofagach (CPS). W literaturze światowej dane na temat związków pomiędzy limfocytami naciekającymi guz nowotworowy a markerami SIR są bardzo skąpe. W CRC dostępne są jedynie pojedyncze doniesienia ze sprzecznymi wnioskami.

Dane z piśmiennictwa potwierdzają zaobserwowaną relację między dłuższym OS a wysoką wartością LMR i niską wartością NLR w grupie pacjentów z rakiem jelita grubego i górnej odbytnicy. W badaniu oceniającym populację pacjentów ograniczoną do chorych z rakiem odbytnicy (w większości odcinka środkowego i dolnego) nie wykazano związku wartości LMR, NLR i PLR z OS. Dane z literatury na temat prognostycznej wartości markerów SIR w populacji pacjentów z miejscowo zaawansowanym rakiem odbytnicy są sprzeczne i w związku z tym mniej klarowne niż w raku jelita grubego i w stadium rozsiewu. W literaturze światowej pacjenci z rakiem jelita grubego i pacjenci rakiem odbytnicy są bardzo często włączani do tej samej grupy i oceniani razem, pomimo zasadniczych różnic między tymi nowotworami, co może prowadzić do błędnych wniosków. Wartość prognostyczna markerów SIR wydaje się być różna w obu tych nowotworach.

W prezentowanych badaniach nie stwierdzono korelacji pomiędzy wartościami LMR, NLR, PLR a markerem CEA. W analizie związków z parametrami kliniczno-patologicznymi zaobserwowano statystycznie znamienną zależność między PLR a zajęciem regionalnych węzłów chłonnych. Nie wykazano innych związków między markerami SIR a wielkością guza nowotworowego lub zaawansowaniem choroby.

Parametry LMR, NLR i PLR oceniane na podstawie trzech badań morfologii krwi obwodowej w prospektywnym badaniu wśród wyselekcjonowanej grupy pacjentów z miejscowo zaawansowanymi rakami odbytnicy (LARC) okazały się umiarkowanie powtarzalne.

Wśród pacjentów z LARC istotnym prognostycznie markerem radiologicznym jest naciekanie naczyń zlokalizowanych poza ścianą odbytnicy (EMVI). EMVI wiąże się z większym ryzykiem wystąpienia przerzutów odległych i krótszym RFS. U pacjentów z EMVI-dodatnim rakiem statystycznie częściej występowały duże guzy z zajęciem regionalnych węzłów chłonnych. Obecność EMVI w MRI przed rozpoczęciem leczenia przedoperacyjnego była związana również z większym zaawansowaniem choroby w histopatologicznym materiale pooperacyjnym. Pacjenci z EMVI-dodatnim rakiem mieli wyższe średnie wartości markera CEA, poziom neutrocytów i trombocytów. Nie przekładało się to jednak na statystycznie istotną zależność między parametrem EMVI a markerami SIR. Związek między zaawansowaniem choroby nowotworowej wg klasyfikacji TNM a obecnością EMVI jest zgodny z danymi z piśmiennictwa. Prezentowana praca jest pierwszym badaniem oceniającym relację pomiędzy markerami SIR a statusem EMVI ocenianym na podstawie MRI u pacjentów z LARC.

Podsumowując, oceniane na podstawie morfologii krwi obwodowej markery ogólnoustrojowej reakcji zapalnej, wydają się być powiązane z zaburzeniami immunologicznymi w mikrośrodowisku guza. To niezwykle interesujące zjawisko, dotychczas nieudokumentowane w literaturze, wymaga potwierdzenia w kolejnych badaniach. Niski koszt i powszechna dostępność oceny wykładników SIR sprawiają, że są one atrakcyjnymi markerami prognostycznymi. Zbiór zaprezentowanych prac jest wstępem do dalszych analiz pozwalających określić ich praktyczne zastosowanie w codziennej praktyce klinicznej.

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Oświadczenia współautorów publikacji wchodzących w skład rozprawy doktorskiej

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- Gawiński, C.; Michalski, W.; Mróz, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Tumor-Infiltrating Lymphocytes (TILs) in Left-Sided Colorectal Cancer Patients. *Biology***2022**, *11*, 385. https://doi.org/10.3390/biology11030385

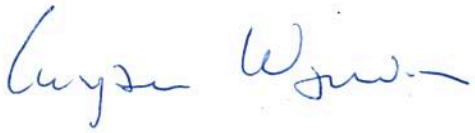
- Gawiński, C.; Mróz, A.; Roszkowska-Purska, K.; Sosnowska, I.; Derezińska-Wołek, E.; Michalski, W., Wyrwicz, L. A Prospective Study on the Roles of the Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), and Platelet-to-Lymphocyte Ratio (PLR) in Patients with Locally Advanced Rectal Cancer. *Biomedicines***2023**, *11*, 3048. https://doi.org/10.3390/biomedicines11113048

- Gawiński, C.; Hołdakowska, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Extramural Vascular Invasion (EMVI) in Locally Advanced Rectal Cancer. *Curr. Oncol.***2023**, *30*, 545-558. https://doi.org/10.3390/curroncol30010043

oświadczam, iż mój udział w powstaniu tych prac polegał na koncepcji i organizacji badań, ocenie i interpretacji wyników, wsparciu merytorycznym i współedycji manuskryptów.

Jednocześnie wyrażam zgodę na przedłożenie powyższych prac przez lek. Cieszymierza Gawińskiego jako elementu rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część powyższych prac wykazuje indywidualny wkład lek. med. Cieszymierza Gawińskiego przy opracowaniu koncepcji badań, opracowywaniu i analizie ich wyników oraz redagowaniu publikacji.



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Oświadczam, iż samodzielna i możliwa do wyodrębnienia część powyższych prac wykazuje indywidualny wkład lek. med. Cieszymierza Gawińskiego przy opracowaniu koncepcji badań, opracowywaniu i analizie ich wyników oraz redagowaniu publikacji.

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oświadczam, iż mój udział w powstaniu pracy polegał na wsparciu merytorycznym, ocenie radiologicznej, analizie badań obrazowych i współredagowaniu mansukryptu.

Jednocześnie wyrażam zgodę na przedłożenie powyższych prac przez lek. Cieszymierza Gawińskiego jako elementu rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część powyższych prac wykazuje indywidualny wkład lek. med. Cieszymierza Gawińskiego przy opracowaniu koncepcji badań, opracowywaniu i analizie ich wyników oraz redagowaniu publikacji.

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- Gawiński, C.; Michalski, W.; Mróz, A.; Wyrwicz, L. Correlation between Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR) and Tumor-Infiltrating Lymphocytes (TILs) in Left-Sided Colorectal Cancer Patients. *Biology***2022**, *11*, 385. https://doi.org/10.3390/biology11030385

- Gawiński, C.; Mróz, A.; Roszkowska-Purska, K.; Sosnowska, I.; Derezińska-Wołek, E.; Michalski, W.; Wyrwicz, L. A Prospective Study on the Roles of the Lymphocyte-to-Monocyte Ratio (LMR), Neutrophil-to-Lymphocyte Ratio (NLR), and Platelet-to-Lymphocyte Ratio (PLR) in Patients with Locally Advanced Rectal Cancer. *Biomedicines***2023**, *11*, 3048. https://doi.org/10.3390/biomedicines11113048

oświadczam, iż mój udział w powstaniu tych prac polegał na wsparciu merytorycznym, przeprowadzaniu i interpretacji analiz statystycznych.

Jednocześnie wyrażam zgodę na przedłożenie powyższych prac przez lek. Cieszymierza Gawińskiego jako elementu rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

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oświadczam, iż mój udział w powstaniu pracy polegał na pomocy organizacyjno-administracyjnej związanej z przeprowadzeniem badań patomorfologicznych.

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oświadczam, iż mój udział w powstaniu pracy polegał na przeprowadzeniu analiz laboratoryjnych.

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