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# PROBLEMY WSPÓŁCZESNEJ MEDYCYNY CZĘŚĆ IV

Redakcja naukowa
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Wydawnictwo Uniwersytetu Medycznego w Łodzi

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## PROBLEMS IN CONTEMPORARY MEDICINE PART IV

Redakcja naukowa

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## Spis treści

UMEDICAL REPORTS

# CHARAKTERYSTYKA CHEMICZNA OLEJKÓW ETERYCZNYCH I FRAKCJI LOTNYCH Z KWIATÓW, LIŚCI I ŁODYG *HELICHRYSUM ARENARIUM* (L.) MOENCH

## CHEMICAL CHARACTERIZATION OF ESSENTIAL OILS AND VOLATILE FRACTIONS FROM THE FLOWERS, LEAVES, AND STEMS OF *HELICHRYSUM ARENARIUM* (L.) MOENCH

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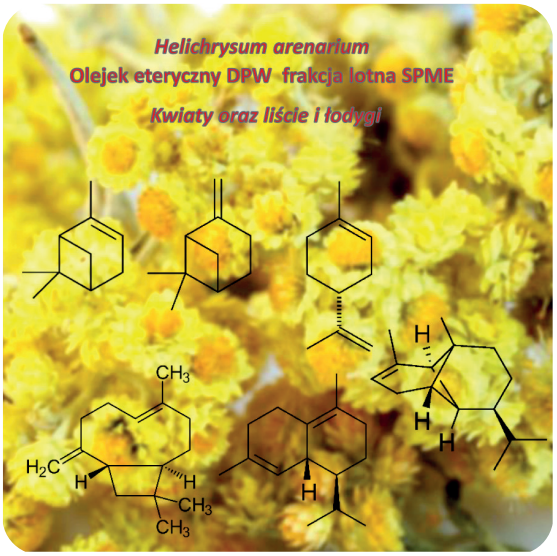

**Streszczenie:** *Helichrysum arenarium* – kocanka piaskowa z rodziny Asteraceae – od wieków znana jest w lecznictwie tradycyjnym. Celem pracy była analiza składu chemicznego olejków eterycznych i frakcji lotnych emitowanych znad kwiatostanów oraz liści i łodyg kocanek zebranych z dwóch naturalnych stanowisk występowania w Polsce. Olejki eteryczne izolowano metodą destylacji z parą wodną (DPW), a frakcje lotne z wykorzystaniem mikroekstrakcji do fazy stałej z fazy nadpowierzchniowej (HS-SPME). Skład chemiczny olejków i frakcji analizowano metodą chromatografii gazowej sprzężonej ze spektrometrią mas (GC-MS). Wyniki wskazały na węglowodory seskwiterpenowe, m.in. (*E*)-*β*-kariofilen i *δ*-kadinen, oraz węglowodory monoterpenowe, takie jak *α*-pineny i *β*-pineny oraz limonen jako dominujące składniki olejków lub frakcji lotnych nadpowierzchniowych. Analiza porównawcza próbek pochodzących z różnych lokalizacji w Polsce ujawniła zbliżony profil chemiczny, z dominującą frakcją składników seskwiterpenowych. Zestawienie wyników z danymi literaturowymi wskazuje na podobieństwa w składzie jakościowym i na różnice w składzie ilościowym terpenów olejków, determinowanych pochodzeniem geograficznym, co podkreśla wpływ warunków środowiskowych na skład i właściwości tych olejków. Występowanie (*E*)-*β*-kariofilenu sugeruje potencjalne działanie przeciwzapalne i przeciwbakteryjne badanych olejków, natomiast limonen i *α*-pinen mogą wykazywać właściwości antyoksydacyjne, co wspiera możliwość zastosowania badanych olejków w kosmetologii i aromaterapii. Zastosowana metoda HS-SPME, w połączeniu z GC-MS, okazała się skuteczna w analizie wysoce lotnych monoterpenów i alifatycznych nieterpenowych składników, wykazując jej przydatność w badaniach nad olejkodajnymi substancjami roślinnymi.

**Słowa kluczowe:** *Helichrysum arenarium*, olejek eteryczny, destylacja z parą wodną, frakcja lotna, HS-SPME

**Abstract:** *Helichrysum arenarium* commonly known as sand immortelle, belonging to the *Asteraceae* family, has been recognized for centuries for its use in traditional medicine. This study aimed to analyze the chemical composition of essential oils and volatile fractions emitted from the flower heads, leaves, and stems of *H. arenarium* collected from two natural habitats in Poland. Essential oils were isolated using steam distillation (DPW), while volatile fractions were extracted using headspace solid-phase microextraction (HS-SPME). The chemical composition of these samples was analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The results revealed the presence of sesquiterpene hydrocarbons, such as (*E*)-*β*-caryophyllene and *δ*-cadinene, and monoterpene hydrocarbons, including *α*- and *β*-pinenes and limonene, as the predominant components of the essential oils or headspace volatile fractions. Comparative analysis of samples from different locations in Poland showed a similar chemical profile, dominated by sesquiterpene fractions. Comparison with literature data highlighted qualitative similarities and quantitative differences in the terpene composition of the oils, driven by geographical origin, thereby underscoring the influence of environmental conditions on the composition and properties of essential oils. The presence of (*E*)-*β*-caryophyllene suggests potential anti-inflammatory and antibacterial properties of the oils, while limonene and *α*-pinene may exhibit antioxidant effects, supporting the potential application of the studied oils in cosmetology and aromatherapy. The HS-SPME method, coupled with GC-MS proved to be effective in analyzing highly volatile monoterpenes and aliphatic non-terpene components, demonstrating its applicability in studies of essential oil-bearing plant materials.

**Keywords:** *Helichrysum arenarium*, essential oil, hydrodistillation, volatile fraction, HS-SPME

**Wykaz skrótów**

**CB1** – receptor kannabinoidowy 1
**CB2** – receptor kannabinoidowy 2
**DPW** – destylacja z parą wodną
**DVB/CAR/PDMS** – diwinylobenzen/ karboksen/polidimetylosiloksan
**GC** – chromatografia gazowa
**HS-SPME** – mikroekstrakcja do fazy stałej z fazy nadpowierzchniowej (ang. *headspace solid phase microextraction)*
**IL-6** – interleukina 6
**Mcz** – masa cząsteczkowa
**MS** – spektrometria mas
**RI** – indeks retencji
**ROS** – reaktywne formy tlenu (ang. *reactive oxygen species)*
**TNF-α** – czynnik martwicy nowotworów alfa

## 1. Wprowadzenie

*Helichrysum arenarium* (L.) Moench, kocanka piaskowa, jest gatunkiem z rodziny Asteraceae, który od średniowiecza znajduje zastosowanie w lecznictwie. W medycynie ludowej roślina ta jest znana pod wieloma nazwami, np. kocanka cytrynowa, nieśmiertelnik żółty czy suchołuska. Nazwa rodzajowa *Helichrysum* wywodzi się od greckich słów nawiązujących do barwy kwiatów: *helios* – słońce, *chrysos* – złoto, natomiast nazwa gatunkowa *arenarium* pochodzi od łacińskiego słowa *arenaris*, co oznacza „piaskowy", „rosnący na piaskach" i odnosi się do naturalnych stanowisk występowania kocanek (Pljevljakušić i in., 2018). Kocanki rosną na obszarach piaszczystych, dobrze nasłonecznionych. Preferują suche łąki, skarpy, pobocza, pola i zarośla (Sawilska, 2015; Pljevljakušić i in., 2018).

*H. arenarium* jest byliną wytwarzającą odrosty korzeniowe oraz kłącza, z których wyrastają liczne rozety liściowe oraz pojedyncze, wznoszące się pędy kwiatostanowe. Cała roślina jest pokryta wełnistym owłosieniem. Ma charakterystyczny, słodkawy, lekko korzenny zapach oraz gorzkawy, ściągający smak. Drobne kwiaty rurkowate o koronie zwykle pomarańczowej są zebrane w kuliste koszyczki, a te w baldachokształtną wiechę. Liczne listki okrywy koszyczka są suche i błoniaste, ułożone dachówkowato, mają najczęściej cytrynowożółte zabarwienie (Sawilska, 2015).

Kwiatostany kocanek zawierają głównie flawonoidy, w tym chalkony z dominującym izosalipurpozydem, flawanony: salipurpozyd oraz inne mono- i diglikozydy naryngeniny, flawonole: mono- i diglikozydy kemferolu i kwercetyny oraz flawony: mono- i diglikozydy apigeniny i luteoliny (Dănăilă-Guidea i in., 2022; Pljevljakušić i in., 2018; Akaberi i in., 2019). Zgodnie z wymaganiami Farmakopei Polskiej XIII kwiaty kocanek – *Helichrysi flos* – powinny zawierać nie mniej niż 0,5% flawonoidów w przeliczeniu na kwercetynę (FP XIII, 2023). Ponadto w składzie kwiatów występują także ftalidy, kumaryny, laktony seskwiterpenowe, olejek eteryczny (0,05%) oraz fitosterole: 3-*O*-glukozydy *β*-sitosterolu i stigmasterolu (Dănăilă-Guidea i in., 2022; Pljevljakušić i in., 2018; Zheljazkov i in., 2022).

Dane literaturowe wskazują, iż związki czynne obecne w kwiatostanach *H. arenarium* wykazują działanie spazmolityczne na mięśnie gładkie pęcherzyka żółciowego, przewodów żółciowych oraz zwieracza bańki wątrobowo-trzustkowej. Dzięki temu przywracają prawidłową amplitudę ruchów perystaltycznych tych struktur. Zmniejszenie oporów dla prądu żółci prowadzi także do jej łatwiejszego i regularniejszego przepływu do dwunastnicy. Preparaty z kwiatostanów kocanek są stosowane w leczeniu różnych schorzeń związanych z układem żółciowym, w tym w atonii pęcherzyka żółciowego, skurczu zwieracza bańki wątrobowo-trzustkowej, w niedostatecznym wytwarzaniu żółci bądź jej utrudnionym przepływie, pomocniczo w kamicy dróg żółciowych oraz po operacjach w obrębie dróg żółciowych (Dănăilă-Guidea i in., 2022; Pljevljakušić i in., 2018; Lamer-Zarawska i in., 2007; Akaberi i in., 2019). Wykorzystanie kwiatów kocanek w lecznictwie jest również udokumentowane przez Europejską Agencję Leków (EMA, 2016). Duża różnorodność składu chemicznego kwiatostanów kocanek sugeruje, że roślina ta może wykazywać szersze niż żółciopędne i wspomagające trawienie właściwości terapeutyczne. W literaturze naukowej udokumentowano także jej działanie przeciwzapalne, przeciwbakteryjne i przeciwgrzybicze (Dănăilă-Guidea i in., 2022; Pljevljakušić i in., 2018; Zheljazkov i in., 2022). Za takie właściwości mogą odpowiadać również składniki olejku eterycznego kocanek piaskowych (Dănăilă-Guidea i in., 2022). W związku z tym celem niniejszej pracy jest przybliżenie charakterystyki składników zapachowych z kwiatostanów oraz niezależnie z liści i łodyg *Helichrysum arenarium*, pozyskanych z dwóch różnych stanowisk jej naturalnego występowania w Polsce. Olejki eteryczne zostały wyizolowane z materiału roślinnego metodą destylacji z parą wodną (DPW), a frakcje lotne znad surowca – metodą mikroekstrakcji do fazy stałej z fazy nadpowierzchniowej (HS-SPME). Olejki oraz frakcje lotne otrzymane z substancji roślinnych analizowano następnie metodą chromatografii gazowej w połączeniu ze spektrometrią mas (GC-MS). Uzyskane wyniki poddano analizie w celu określenia jakościowego i ilościowego składu frakcji związków lotnych, odpowiedzialnych m.in. za zapach analizowanych substancji roślinnych *H. arenarium*. Porównano również skład chemiczny badanych olejków eterycznych w zależności od regionu geograficznego występowania rośliny.

## 2. Materiały i metody

### *2.1. Materiał roślinny*

Materiał do badań stanowiły kwiatostany oraz łącznie liście i łodygi *Helichrysum arenarium* (L.) Moench zebrane z dwóch stanowisk naturalnego występowania tego gatunku, tj. z piaszczystych nieużytków w Gorodzisku (52°50´N 23°35´E, woj. podlaskie) i z niezagospodarowanej działki budowlanej w Kęblinach (51°55´N 198°30´E, woj. łódzkie). Wymienione stanowiska zbioru materiału roślinnego dzieliła znaczna odległość – 376 km. Identyfikacja gatunku *H. arenarium* została przeprowadzona przez autorów niniejszej pracy we współpracy ze specjalistami w dziedzinie taksonomii roślin z Ogrodu Roślin Leczniczych Uniwersytetu Medycznego w Łodzi.

Po zbiorze świeżego materiału roślinnego rozdrobniono go ręcznie, a następnie, po zważeniu, przeznaczono do izolacji olejków eterycznych oraz frakcji lotnych z wykorzystaniem odpowiednio metod DPW i HS-SPME.

### *2.2. Destylacja z parą wodną (DPW)*

Olejki eteryczne z kwiatostanów oraz niezależnie z liści i łodyg *H. arenarium* izolowano metodą destylacji z parą wodną (DPW) z wykorzystaniem aparatu Derynga (Rassem i in., 2016; FP VI, 2002). Do 400 g kwiatostanów i 288 g liści i łodyg zebranych w Gorodzisku oraz 1470 g kwiatostanów i 1000 g liści i łodyg zebranych w Kęblinach dodano 3 l wody. Otrzymaną mieszaninę poddano trzygodzinnej destylacji z parą wodną. Uzyskane olejki eteryczne zbierano w odbieralniku, w mieszaninie pentan–eter dietylowy (1:1), wysuszono bezwodnym siarczanem (VI) magnezu i przechowywano w temperaturze 4°C do czasu przeprowadzenia analizy GC-MS.

### *2.3. Mikroekstrakcja do fazy stałej z fazy nadpowierzchniowej (HS-SPME)*

W celu izolacji frakcji związków lotnych emitowanych znad surowca zastosowano metodę HS-SPME (Pawliszyn, 1997; Agatonovic-Kustrin i in., 2023). W tym celu 3,5 g kwiatostanów i 4,9 g liści i łodyg zebranych w Gorodzisku oraz 3,5 g kwiatostanów i 5,0 g liści i łodyg pozyskanych w Kęblinach umieszczono w szczelnie zamkniętych szklanych fiolkach, które następnie przeniesiono do łaźni wodnej (temp. 60°C) i termostatowano przez 10 min. Po ustaleniu równowagi między matrycą a fazą gazową wprowadzono do fiolki włókno sorpcyjne SPME pokryte fazą DVB/CAR/PDMS (diwinylobenzen/karboksen/polidimetylosiloksan) o grubości 60 µm (Supelco) i prowadzono ekstrakcję składników lotnych przez 30 min. W celu desorpcji badanych analitów włókno niezwłocznie wprowadzono do dozownika chromatografu gazowego GC (280°C) na 2 min.

### *2.4. Analiza GC-MS*

Analizy GC-MS składników olejkowych i frakcji lotnych wykonano przy użyciu chromatografu gazowego Trace GC Ultra sprzężonego ze spektrometrem masowym DSQ II (Thermo Electron) z wykorzystaniem niepolarnej kolumny kapilarnej Rtx-1MS (Restek) o wymiarach 60 m × 0,25 mm i grubości filmu 0,25 µm. Temperatura kolumny zaprogramowana w zakresie: 50–300°C, z przyrostem liniowym 4°C/min. Temperatura dozownika – 280°C, detektora – 300°C. Gaz nośny – hel z przepływem 0,8 ml/min, ciśnienie stałe – 300 kPa, energia jonizacji – 70 eV, temperatura źródła jonów – 200°C.

Identyfikację składu olejków eterycznych i frakcji lotnych przeprowadzono przez porównanie indeksów retencji (RI), ustalonych na podstawie czasów retencji mieszaniny wzorcowych *n*-alkanów C8-C32 z indeksami literaturowymi (Babushok i in., 2011) i widm masowych MS analizowanych składników z widmami MS wzorców z bibliotek widm NIST 98.1 Mass Finder 3.1., dostępnych w programie Xcalibur (Thermo Electron Corporation).

## 3. Wyniki

### *3.1. Skład chemiczny olejków eterycznych H. arenarium*

Olejki eteryczne *H. arenarium* otrzymane metodą DPW z kwiatostanów oraz łącznie z liści i łodyg zebranych w Gorodzisku (woj. podlaskie) charakteryzowały się lekko niebieską barwą, natomiast olejki uzyskane z tych samych surowców zebranych w Kęblinach (woj. łódzkie) były koloru jasnożółtego. Wszystkie olejki cechowały się aromatycznym, lekko ziołowym zapachem.

Analiza metodą GC-MS olejków eterycznych z kwiatostanów pochodzących z Gorodziska i Kęblin wykazała obecność odpowiednio 94 i 96 związków, stanowiących 94,0% i 93,2% wszystkich składników olejkowych. Z kolei w olejkach z liści i łodyg, pozyskanych z tych samych stanowisk, wykryto odpowiednio 72 i 73 związki odpowiadające 88,4% i 89,6% sumy składników. W kwiatowych olejkach dominującymi składnikami były *β*-pinen, (*E*)-*β*-kariofilen i *δ*-kadinen, natomiast w olejkach z liści i łodyg przeważały (*E*)-*β*-kariofilen, *α*-pinen oraz *δ*-kadinen lub limonen (Tabela 1).

Każdy z olejków kwiatowych zawierał 12 węglowodorów monoterpenowych, stanowiących 17,1% i 17,4% całkowitej zawartości olejków, odpowiednio z Gorodziska i Kęblin. W olejkach z liści i łodyg liczba węglowodorów monoterpenowych wynosiła odpowiednio 11 i 10 związków, stanowiących kolejno 13,8% i 23,3% składu. Główne składniki tej grupy obejmowały *α*- i *β*-pineny (2,9–8,8%) oraz limonen (2,3–5,2%), które łącznie stanowiły ponad 75% całkowitej zawartości monoterpenów. Kolejna grupa licznych składników olejkowych to tlenowe pochodne monoterpenów. W olejkach kwiatowych wykryto po 15 i 16 związków tej grupy, stanowiących 9,5% i 11,5% składu, odpowiednio z Gorodziska i Kęblin. W olejkach z liści i łodyg wykryto 11 i 15 składników, których udział wynosił 5,9% i 10,6%. Spośród tej grupy związków na uwagę zasługują: *α*-terpineol (2,0–3,7%), 1,8-cyneol (1,4–3,3%), linalol (1,0–2,0%) i borneol (0,5–1,4%).

Węglowodory seskwiterpenowe stanowiły główną frakcję lotną wszystkich analizowanych olejków eterycznych z *H. arenarium*. W olejkach kwiatowych wykryto 22 i 20 związków tej grupy, stanowiących odpowiednio 33,2% i 33,0% wszystkich składników olejków, odpowiednio z Gorodziska i Kęblin. W olejkach z liści i łodyg zidentyfikowano po 21 związków, stanowiących odpowiednio 42,4% i 28,9% składu. Dominowały tutaj bicykliczne seskwiterpeny o szkielecie kadinanu, m.in. *α*- i *γ*-muuroleny, *α*-, *γ*- i *δ*-amorfeny oraz *α*-, *γ*- i *δ*-kadineny. Ich sumaryczny udział wynosił 39,3–49,6% składu zidentyfikowanych seskwiterpenów. Ponadto obecne były w olejkach seskwiterpeny m.in. o szkielecie aromadendranu: aromadendren i *allo*-aromadendren, gwajanu: *β*-gwajen, kariofilanu: (*E*)-*β*-kariofilen, izokariofilen i humulanu: *α*-humulen oraz seskwiterpeny acykliczne: (*E*)-*β*-farnezen. Spośród indywidualnych węglowodorów seskwiterpenowych dominowały w olejkach: (*E*)-*β*-kariofilen (7,4–11,4%), *δ*-kadinen (3,4–5,6%) oraz (*E*)-*β*-farnezen (1,3–4,7%). Tlenowe pochodne seskwiterpenów również były licznie reprezentowane w analizowanych olejkach. W olejkach kwiatowych z Gorodziska i Kęblin zidentyfikowano 10 i 13 takich związków (odpowiednio 10,8% i 12,0% składu), natomiast w olejkach z liści i łodyg odpowiednio 12 i 11 związków, które stanowiły 20,7% i 17,6%. Do dominujących związków tej grupy należały: *τ*-kadinol (2,5–5,3%), *α*-kadinol (1,3–2,6%) oraz tlenek (*E*)-*β*-kariofilenu (1,0–2,5%).

Olejki eteryczne zawierały również związki o budowie alifatycznych nieterpenów. W olejkach kwiatowych z Gorodziska i Kęblin zidentyfikowano odpowiednio 28 i 30 związków z tej grupy, stanowiących 21,1% i 16,5% składu, a w olejkach z liści i łodyg – odpowiednio 10 i 11 związków, stanowiących 3,3% i 7,9%. Dominujące związki tej grupy to dekanal (do 3,5%), (*E*)-heks-2-enal (do 3,5%) oraz nonanal (do 2,7%).

W badanych olejkach stwierdzono także występowanie innych, rzadziej spotykanych związków, jak chamazulen (0,1–1,4%), który nadaje olejkom charakterystyczną, niebieską barwę. Kolejnym składnikiem jest eugenol z grupy fenylopropanoidów, a także *β*-damascenon i *β*-damaskon, związki należące do nienasyconych ketonów różanych.

*3.2. Skład chemiczny frakcji lotnych H. arenarium*

Frakcje lotne z kwiatostanów oraz łącznie z liści i łodyg *H. arenarium* zebranych w Gorodzisku i Kęblinach otrzymano metodą mikroekstrakcji do fazy stałej z fazy nadpowierzchniowej (HS-SPME). Metoda ta jest ceniona w analizie lotnych związków organicznych, umożliwia ich bezrozpuszczalnikowe pobieranie bezpośrednio znad surowca i koncentrację, co pozwala na precyzyjną analizę przy minimalnej ingerencji w próbkę. W trakcie tego procesu związki lotne adsorbują się na włóknie SPME, a następnie są desorbowane w wysokiej temperaturze w dozowniku chromatografu gazowego (GC) (Pawliszyn, 1997; Agatonovic-Kustrin i in., 2023).

Analiza GC-MS frakcji lotnych z kwiatostanów z Gorodziska i Kęblin wykazała obecność 43 i 42 związków, stanowiących odpowiednio 91,9% i 86,9% składu (Tabela 1). Natomiast we frakcjach lotnych z liści i łodyg wykryto odpowiednio 40 i 44 składniki, których zawartość wynosiła 89,3% i 92,8%. Frakcje lotne z kwiatostanów, niezależnie od miejsca pochodzenia, charakteryzowały się przewagą (*E*)-*β*-kariofilenu, limonenu i *α*-pinenu. Natomiast we frakcjach z liści i łodyg dominowały limonen oraz *α*-pinen lub (*E*)-*β*-kariofilen.

W lotnych frakcjach z kwiatostanów, niezależnie od miejsca ich zbioru, dominowały węglowodory monoterpenowe i seskwiterpenowe. Z monoterpenów zidentyfikowano 13 i 11 węglowodorów stanowiących odpowiednio 37,6% i 40,1% składu, z dominującymi limonenem (13,4–15,7%), *α*-pinenem (9,9–10,3%) i *β*-pinenem (5,6–6,2%). Z kolei z seskwiterpenów oznaczono 16 i 19 składników z ich udziałem na poziomie 41,4% i 39,5%, gdzie głównymi związkami były (*E*)-*β*-kariofilen (14,8–18,1%), *α*-kopaen (5,6–6,4%) oraz *δ*-kadinen (2,9–4,5%).

Frakcje składników lotnych z liści i łodyg były najbogatsze w węglowodory monoterpenowe. Zidentyfikowano kolejno 11 i 12 związków tej grupy, stanowiące odpowiednio 46,0% i 42,4% sumy oznaczonych składników, z dominującymi limonenem (17,9–19,9%), *α*-pinenem (10,5–12,5%) i *β*-pinenem (2,4–6,8%). W znaczącej ilości występowały również węglowodory seskwiterpenowe z 18 i 16 zidentyfikowanymi składnikami tej grupy, stanowiącymi 25,6% i 30,1% składu i przeważającymi (*E*)-*β*-kariofilenem (5,6–13,8%) oraz *α*-kopaenem (5,2–5,9%).

Porównanie składu chemicznego wyżej scharakteryzowanych olejków eterycznych oraz frakcji lotnych z kwiatostanów oraz z liści i łodyg *H. arenarium* z Gorodziska i Kęblin (Tabela 1) wskazuje, iż węglowodory seskwiterpenowe dominowały zarówno w olejkach eterycznych, jak i w niektórych frakcjach lotnych. Węglowodory monoterpenowe przeważały głównie we frakcjach lotnych, ze względu na wyższy poziom ich adsorpcji na włóknach SPME. Z kolei tlenowe pochodne monoterpenów oraz węglowodory alifatyczne mogły słabiej wiązać się z włóknem, co skutkowało ich znacznie niższą zawartością we frakcjach lotnych. Warto również podkreślić, że tlenowe pochodne seskwiterpenów, o niemal dwukrotnie większej masie molowej i polarności od monoterpenów, nie zostały wyizolowane metodą HS-SPME z badanych próbek roślinnych. Natomiast w fazie nadpowierzchniowej surowców, zastosowanie metody HS-SPME umożliwiło identyfikację specyficznych składników determinujących zapach kocanki, m.in. heksanalu, związku przypominającego zapach świeżej trawy z nutą owocową.

**Tabela 1.** Wyniki analizy GC-MS składu chemicznego olejków eterycznych oraz frakcji lotnych otrzymanych z kwiatostanów oraz liści i łodyg *H. arenarium*.

| składnik | RI | Mcz | Olejek eteryczny DPW | | | | Frakcja lotna HS-SPME | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | kwiaty [a] | kwiaty [b] | liście łodygi [a] | liście łodygi [b] | kwiaty [a] | kwiaty [b] | liście łodygi [a] | liście łodygi [b] |
| | | | % | | | | | | | |
| heksanal | 778 | 100 | - | | - | - | 0.5 | 0.5 | 2.0 | 0.7 |
| oktan | 799 | 114 | 0.2 | 0.1 | 0.1 | 0.1 | - | - | - | - |
| (*E*)-heks-2-enal | 830 | 98 | - | - | 0.4 | 3.5 | - | - | 0.5 | 0.4 |
| non-1-en | 850 | 126 | - | - | 0.2 | - | - | - | - | - |
| (*E*)-heks-3-en-1-ol | 843 | 100 | - | - | - | 0.9 | - | - | - | - |
| heksan-1-ol | 857 | 102 | - | - | - | 1.5 | - | - | - | - |
| heptanal | 882 | 114 | 0.2 | 0.3 | - | 0.3 | - | - | - | - |
| nonan | 889 | 128 | 0.3 | 0.2 | - | - | - | - | - | - |
| 4-metyloheptan-3-on | 916 | 128 | 1.8 | 0.5 | - | - | 3.8 | 0.5 | 7.9 | 0.6 |
| *α*-pinen | 931 | 136 | 4.3 | 4.0 | 5.0 | 8.8 | 9.9 | 10.2 | 10.5 | 12.5 |
| *α*-fenchen | 942 | 136 | - | - | - | - | - | 0.1 | 0.2 | 0.2 |
| kamfen | 943 | 136 | 0.6 | 0.6 | 0.4 | 1.1 | 1.0 | 0.7 | 0.9 | 1.0 |
| 4-metyloheptan-3-ol | 956 | 130 | 0.1 | - | - | - | - | - | - | - |
| 6-metylohept-5-en-2-on | 964 | 126 | - | - | - | - | 0.7 | 0.3 | 1.3 | 0.4 |
| *β*-pinen | 970 | 136 | 8.0 | 7.2 | 2.9 | 4.3 | 6.2 | 5.6 | 6.8 | 2.4 |
| 2-pentylofuran | 978 | 138 | 1.3 | 1.2 | 0.3 | 0.6 | - | - | - | - |
| heptanian metylu | 979 | 144 | - | - | - | - | - | 1.9 | - | 1.7 |
| oktanal | 981 | 128 | 1.9 | 1.8 | - | - | - | - | - | - |
| mircen | 981 | 136 | - | - | 1.0 | 1.6 | 2.1 | 2.6 | 3.2 | 2.5 |
| izolimonen | 985 | 136 | 0.6 | 0.4 | 0.3 | 0.4 | - | - | - | - |
| 2,3-dehydro-1,8-cyneol | 993 | 152 | - | - | 0.2 | 0.2 | - | - | - | - |
| *α*-felandren | 993 | 136 | 0.1 | 0.1 | - | - | 0.2 | 0.2 | 0.4 | 0.2 |
| *p*-metyloanizol | 997 | 122 | - | 0.4 | - | - | - | - | - | - |
| *δ*-kar-3-en | 999 | 136 | 0.2 | - | - | - | - | - | - | - |
| *α*-terpinen | 1005 | 136 | - | 0.2 | - | 0.1 | 0.5 | 0.5 | 0.2 | 0.3 |
| *m*-cymen | 1009 | 134 | 0.2 | 0.3 | 0.1 | 0.5 | 0.6 | 0.8 | 1.5 | 1.3 |
| 1,8-cyneol | 1016 | 154 | 1.6 | 3.3 | - | 1.4 | - | - | - | 11.7 |
| limonen | 1019 | 136 | 2.3 | 3.2 | 3.1 | 5.2 | 13.4 | 15.7 | 19.9 | 17.9 |
| (*Z*)-*β*-ocymen | 1028 | 136 | 0.1 | 0.1 | 0.2 | - | 0.5 | - | - | - |
| (*E*)-*β*-ocymen | 1039 | 136 | 0.1 | 0.3 | 0.1 | - | 0.3 | - | - | - |
| *γ*-terpinen | 1050 | 136 | 0.3 | 0.5 | 0.5 | 1.0 | 1.2 | 2.2 | 1.8 | 2.8 |
| oktan-1-ol | 1060 | 130 | 0.3 | 0.4 | - | - | - | - | - | - |
| *p*-cymenen | 1073 | 132 | - | - | - | - | 0.2 | - | - | 0.2 |
| nonan-2-on | 1074 | 142 | - | 0.1 | - | - | - | - | - | - |
| terpinolen | 1079 | 136 | 0.3 | 0.5 | 0.2 | 0.4 | 1.5 | 1.3 | 0.5 | 1.1 |
| nonanal | 1085 | 142 | 2.7 | 2.4 | - | 0.4 | 2.0 | 0.7 | - | 0.2 |
| *β*-linalol | 1086 | 154 | - | - | 2.0 | 1.0 | - | - | - | - |
| *α*-fenchol | 1098 | 154 | 0.4 | 0.6 | 0.2 | 0.3 | 0.2 | 0.2 | - | 0.2 |
| perillen | 1107 | 150 | - | 0.5 | - | - | - | - | - | - |
| *α*-kamfolenal | 1104 | 152 | - | - | - | 0.2 | - | - | - | - |
| kamfora | 1121 | 152 | - | 0.1 | - | - | - | - | - | - |
| *trans*-pinokarweol | 1124 | 152 | 0.2 | 0.1 | - | 0.2 | - | - | - | - |
| izopulegol | 1131 | 154 | 0.4 | 0.4 | - | - | - | - | - | - |
| hydrat kamfenu | 1133 | 154 | - | - | - | 0.3 | - | - | - | 0.1 |
| (*E*)-non-2-enal | 1141 | 140 | 0.4 | 0.4 | - | - | - | - | - | - |
| *δ*-terpineol | 1148 | 152 | - | - | - | - | - | - | - | 0.2 |
| borneol | 1151 | 154 | 1.0 | 1.4 | 0.5 | 1.4 | - | - | - | - |
| kwas kaprylowy | 1157 | 144 | - | - | - | - | 0.5 | 0.2 | - | - |

**Tabela 1**. Wyniki analizy GC-MS składu chemicznego olejków eterycznych… (cd.)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| terpinen-4-ol | 1164 | 154 | 0.7 | 0.8 | 0.2 | 0.4 | 1.0 | 0.6 | 0.2 | 0.5 |
| myrtenal | 1167 | 150 | 0.5 | 0.1 | 0.1 | 0.1 | - | - | - | - |
| *α*-terpineol | 1175 | 154 | 2.6 | 3.0 | 2.0 | 3.7 | 2.1 | 1.9 | 0.6 | 2.5 |
| kaprylan etylu | 1183 | 172 | - | 0.7 | - | - | - | - | - | - |
| dekanal | 1187 | 156 | 3.5 | 2.6 | 0.6 | - | 0.7 | - | - | - |
| werbebon | 1183 | 150 | 0.6 | - | - | - | - | - | - | - |
| *β*-cyklocytral | 1197 | 152 | 0.2 | 0.1 | 0.2 | 0.2 | - | - | 0.2 | 0.1 |
| octan fenchylu | 1205 | 196 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | - | - | 0.1 |
| 2-metylobutanian heksenylu | 1218 | 184 | - | - | - | - | - | - | 3.1 | - |
| 2-metylobutanian heksylu | 1226 | 186 | - | - | - | - | - | - | 1.0 | - |
| (*E*)-dec-2-enal | 1244 | 154 | - | 0.1 | - | - | - | - | - | - |
| aldehyd perilla | 1254 | 150 | 0.4 | 0.5 | 0.4 | 0.9 | - | 0.3 | 0.6 | 0.5 |
| octan bornylu | 1268 | 196 | 0.3 | 0.4 | 0.1 | 0.3 | 0.6 | 0.1 | 0.2 | 0.4 |
| octan lawandulylu | 1273 | 196 | - | 0.1 | - | - | - | - | - | - |
| undekan-2-on | 1276 | 152 | - | 0.2 | - | - | - | - | - | - |
| octan *trans*-pinokarweolu | 1279 | 194 | 0.3 | 0.3 | - | - | 0.3 | - | - | - |
| dihydroedulan | 1280 | 194 | - | - | 0.1 | - | - | - | - | - |
| octan terpinen-4-ylu | 1286 | 196 | 0.2 | 0.2 | - | - | - | - | - | - |
| undekanal | 1289 | 170 | 0.5 | 0.4 | - | - | - | - | - | - |
| tridekan | 1298 | 184 | 0.2 | 0.2 | - | - | 0.3 | 0.2 | - | - |
| eugenol | 1337 | 164 | 0.4 | 0.5 | - | - | - | - | - | - |
| *β*-damascenon | 1362 | 190 | 0.2 | 0.3 | 0.2 | 0.2 | - | - | - | - |
| *α*-ylangen | 1369 | 204 | 0.8 | 0.4 | 0.7 | 0.9 | 1.4 | 1.1 | 1.3 | 0.8 |
| *α*-kopaen | 1374 | 204 | 3.7 | 2.9 | 3.8 | 2.2 | 6.4 | 5.6 | 5.9 | 5.2 |
| kwas kaprynowy | 1379 | 172 | - | 1.0 | - | - | - | - | - | - |
| *β*-bourbonen | 1381 | 204 | - | - | 0.4 | 0.2 | - | - | 0.2 | 0.1 |
| dodekanal | 1389 | 184 | 0.4 | 0.3 | - | - | - | - | - | - |
| tetradekan | 1397 | 198 | 0.8 | 0.7 | - | - | - | - | - | - |
| *β*-damaskon | 1391 | 192 | - | - | 0.2 | 0.1 | - | - | - | - |
| izokariofilen | 1398 | 204 | - | - | - | 0.2 | - | - | - | - |
| longifolen | 1406 | 204 | 0.2 | - | 0.2 | 0.2 | - | - | - | - |
| (*E*)-*β*-kariofilen | 1417 | 204 | 7.4 | 11.4 | 8.3 | 8.6 | 14.8 | 18.1 | 5.6 | 13.8 |
| aristol-1(10)-en | 1424 | 204 | 0.4 | 0.4 | 0.3 | - | - | - | - | - |
| *β*-kopaen | 1426 | 204 | - | - | - | - | 0.4 | 0.4 | - | - |
| geranyloaceton | 1429 | 196 | 0.2 | - | - | 0.3 | - | - | - | - |
| aromadendren | 1435 | 204 | 0.4 | 0.4 | 1.0 | 0.6 | 0.7 | 0.6 | 0.5 | 0.4 |
| (*E*)-*β*-farnezen | 1446 | 204 | 1.3 | 1.4 | 4.7 | 2.8 | 0.7 | 1.6 | 1.0 | 1.6 |
| *α*-humulen | 1448 | 204 | 1.2 | 0.9 | - | - | 0.8 | 0.5 | 0.2 | 0.8 |
| *allo*-aromadendren | 1455 | 204 | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 | 0.2 | 0.1 | - |
| *β*-jonon | 1463 | 192 | 0.1 | - | 0.2 | 0.2 | - | - | - | - |
| *γ*-muurolen | 1469 | 204 | 2.6 | 2.2 | 3.4 | 1.8 | 4.1 | 2.5 | 2.6 | 1.8 |
| *α*-amorfen | 1472 | 204 | 0.8 | 0.7 | 1.3 | 0.6 | 0.7 | 0.5 | 0.5 | 0.3 |
| *β*-gwajen | 1480 | 204 | 0.3 | 0.3 | 0.4 | - | - | - | - | - |
| *γ*-amorfen | 1484 | 204 | 0.7 | 0.6 | 1.0 | 0.5 | 0.9 | 0.6 | 0.7 | 0.4 |
| *epi*-zonaren | 1489 | 204 | - | - | - | - | - | 0.5 | - | - |
| *α*-muurolen | 1491 | 204 | 2.3 | 2.0 | 2.8 | 1.6 | 2.4 | 1.3 | 1.5 | 1.2 |
| *δ*-amorfen | 1497 | 204 | 0.4 | 0.3 | - | 0.3 | - | - | - | - |
| *γ*-kadinen | 1504 | 204 | 2.4 | 2.1 | 3.3 | 1.9 | 2.3 | 1.8 | 2.0 | 1.3 |
| *cis/trans*-kalamenen | 1507 | 202 | 0.7 | 0.6 | 1.3 | 0.8 | 0.4 | 0.3 | 0.4 | 0.3 |
| *δ*-kadinen | 1513 | 204 | 5.5 | 4.5 | 5.6 | 3.4 | 4.5 | 2.9 | 2.2 | 1.6 |
| zonaren | 1515 | 204 | - | - | - | - | - | 0.5 | 0.2 | 0.2 |
| kadina-1,4-dien | 1522 | 204 | 0.6 | 0.5 | 0.8 | 0.4 | - | 0.3 | 0.3 | - |
| *α*-kalakoren | 1528 | 200 | 1.1 | 1.0 | 1.6 | 1.0 | | | | |
| *α*-kadinen | 1528 | 204 | | | | | 0.7 | 0.5 | 0.5 | 0.3 |

**Tabela 1**. Wyniki analizy GC-MS składu chemicznego olejków eterycznych… (cd.)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (*E*)-nerolidol | 1547 | 222 | 0.5 | 0.5 | 0.7 | 0.5 | - | - | - | - |
| kariofilenol | 1558 | 222 | - | 0.4 | 0.6 | 0.8 | - | - | - | - |
| kwas laurynowy | 1561 | 200 | 0.8 | 0.6 | - | - | - | - | - | - |
| tlenek (*E*)-*β*-kariofilenu | 1568 | 220 | 1.0 | 1.9 | 1.9 | 2.5 | - | - | - | - |
| gleenol | 1573 | 222 | 0.8 | 1.3 | 1.4 | - | - | - | - | - |
| wiridiflorol | 1580 | 222 | 0.3 | 0.4 | 0.5 | 1.3 | - | - | - | - |
| tlenek humulenu I | 1592 | 206 | - | - | 1.0 | - | - | - | - | - |
| tetradekanal | 1591 | 212 | 0.9 | - | - | - | - | - | - | - |
| tlenek humulenu II | 1592 | 220 | - | 0.3 | - | - | - | - | - | - |
| heksadekan | 1595 | 226 | 0.4 | - | - | - | - | - | - | - |
| rosifoliol | 1593 | 222 | - | 1.0 | - | 1.6 | - | - | - | - |
| tlenek ledenu | 1602 | 220 | 1.1 | 0.8 | 1.8 | 1.4 | - | - | - | - |
| muurola-4,10(14)-dien-1-ol | 1609 | 220 | 0.7 | 0.5 | 2.3 | 1.4 | - | - | - | - |
| 1,10-di-*epi*-kubenol | 1612 | 222 | - | - | 1.5 | 1.0 | - | - | - | - |
| 1-*epi*-kubenol | 1613 | 222 | 0.8 | 0.7 | 1.1 | 0.8 | - | - | - | - |
| kubenol | 1617 | 222 | 0.5 | 0.4 | - | - | - | - | - | - |
| *τ*-kadinol | 1625 | 222 | 2.9 | 2.5 | 5.3 | 3.9 | - | - | - | - |
| *α*-kadinol | 1638 | 222 | 2.1 | 1.3 | 2.6 | 2.6 | - | - | - | - |
| kadalen | 1656 | 198 | 0.1 | - | 1.3 | 0.5 | - | - | - | - |
| pentadekanal | 1692 | 226 | 1.6 | 0.9 | 0.9 | 0.5 | - | - | - | - |
| chamazulen | 1712 | 184 | 0.1 | - | 1.4 | - | - | - | - | - |
| kwas mirystynowy | 1760 | 228 | 2.1 | 1.4 | - | 0.1 | - | - | - | - |
| heksadekanal | 1795 | 240 | 0.3 | - | - | - | - | - | - | - |
| octadekan | 1795 | 254 | - | 0.2 | - | - | - | - | - | - |
| heksahydroksyfarnezyloaceton | 1827 | 268 | 0.4 | 0.4 | 0.8 | 0.6 | - | - | - | - |
| kwas pentadekanowy | 1838 | 242 | - | 0.1 | - | - | - | - | - | - |
| heptadekanal | 1895 | 254 | 0.2 | 0.2 | 0.1 | - | - | - | - | - |
| kwas palmitynowy | 1956 | 256 | 0.2 | - | 0.1 | - | - | - | - | - |
| palmitynian etylu | 1975 | 284 | - | 0.3 | - | - | - | - | - | - |
| eikozan | 1996 | 282 | 0.1 | 0.1 | - | - | 0.2 | - | - | - |
| oktadekanal | 2000 | 268 | 0.1 | - | - | - | - | - | - | - |
| geranyllinalolu | 2011 | 272 | 0.1 | - | 0.1 | - | - | - | - | - |
| fitol | 2100 | 296 | - | - | 0.1 | 0.1 | - | - | - | - |
| trikozan | 2296 | 324 | 0.2 | 0.1 | - | - | - | - | - | - |
| pentakozan | 2499 | 352 | 0.5 | 0.2 | 0.1 | 0.1 | - | - | - | - |
| heptakozan | 2692 | 380 | 0.2 | 0.1 | - | - | - | - | - | - |
| | | | **94.0** | **93.2** | **88.4** | **89.6** | **91.9** | **86.9** | **89.3** | **92.8** |
| węglowodory monoterpenowe | | | 17.1 | 17.4 | 13.8 | 23.3 | 37.6 | 40.1 | 46.0 | 42.4 |
| tlenowe pochodne monoterpenów | | | 9.5 | 11.5 | 5.9 | 10.6 | 4.2 | 3.0 | 1.8 | 16.4 |
| węglowodory seskwiterpenowe | | | 33.2 | 33.0 | 42.4 | 28.9 | 41.4 | 39.5 | 25.6 | 30.1 |
| tlenowe pochodne seskwiterpenów | | | 10.8 | 12.0 | 20.7 | 17.6 | - | - | - | - |
| węglowodory alifatyczne | | | 21.1 | 16.5 | 3.3 | 7.9 | 8.7 | 4.3 | 15.9 | 3.9 |
| inne | | | 2.5 | 2.7 | 2.3 | 1.3 | - | - | - | - |

Substancje roślinne pozyskane ze stanowisk naturalnego występowania *H. arenarium* w Polsce: (a) – Gorodzisko (woj. podlaskie), (b) – Kębliny (woj. łódzkie), DPW – destylacja z parą wodną, HS-SPME – mikroekstrakcja do fazy stałej z fazy nadpowierzchniowej.

## 4. Dyskusja

Kocanka piaskowa (*Helichrysum arenarium*) jest znana ze swoich dobrze udokumentowanych właściwości leczniczych, szczególnie cenionych w terapii dolegliwości związanych z układem żółciowym (Dănăilă-Guidea i in., 2022; Pljevljakušić i in. 2018). Roślina ta występuje naturalnie w Europie, szczególnie na terenach piaszczystych, i wyróżnia się żółtymi kwiatami oraz unikalnym składem chemicznym (Sawilska, 2015; Pljevljakušić i in., 2018). W niniejszej pracy przeanalizowano skład chemiczny olejków eterycznych otrzymanych z kwiatostanów oraz liści i łodyg kocanek piaskowych zebranych z dwóch naturalnych stanowisk ich występowania w Polsce. Wskazano, iż olejki eteryczne i frakcje lotne, w zależności od materiału surowcowego, charakteryzują się przewagą węglowodorów seskwiterpenowych, w tym (*E*)-*β*-kariofilenu i *δ*-kadinenu, oraz węglowodorów monoterpenowych, m.in. *α*- i *β*-pinenu i limonenu. Zasadne wydaje się więc zestawienie uzyskanych wyników z rezultatami badań nad olejkami eterycznymi tego gatunku prowadzonymi w innych krajach, co pozwoli na lepsze zrozumienie wpływu czynników geograficznych na ich skład chemiczny. W badaniach nad kwiatami z Litwy, Włoch czy Turcji dominującą frakcję olejków eterycznych również stanowiły węglowodory seskwiterpenowe, szczególnie (*E*)-*β*-kariofilen, co potwierdza uniwersalność tego związku jako charakterystycznego składnika olejkowego kwiatów *H. arenarium*, niezależnie od regionu pochodzenia (Judžentienė i Butkienė, 2006; Reidel i in., 2017; Tığlı Kaytanlıoğlu i in., 2021). Wyjątek w badaniach stanowiły jedynie kwiaty z Chin, gdzie dominującym składnikiem olejku był alkohol seskwiterpenowy, tj. *β*-spatulenol (Liu i in., 2019). Różnice mogą wynikać z adaptacji kocanki piaskowej do specyficznych warunków środowiskowych Chin, w tym większego nasłonecznienia oraz ekstremalnych wahań temperatury. Natomiast w przypadku porównania składu olejków otrzymanych z liści kocanek z Litwy odnotowano wyższy udział *δ*-kadinenu, co może wskazywać na wpływ specyficznych warunków klimatycznych tego kraju, takich jak wyższa wilgotność i umiarkowana temperatura, które sprzyjają biosyntezie tego związku (Judžentienė i Butkienė, 2006). W olejku z liści z Czarnogóry, pomimo nadal wyraźnie dominującej frakcji węglowodorów seskwiterpenowych, wykazano najwyższy udział di-*epi*-*α*-cedrenu, co może być efektem gorętszego, śródziemnomorskiego klimatu tego regionu (Rančić i in., 2005).

W kontekście zastosowania olejków eterycznych *H. arenarium* w lecznictwie, jednym z kluczowych związków jest (*E*)-*β*-kariofilen, seskwiterpen o przyjemnym, goździkowo-terpentynowym zapachu. Związek ten wykazuje silne działanie przeciwbakteryjne, hamuje wzrost bakterii Gram-dodatnich i Gram-ujemnych, w tym m.in. *Staphylococcus aureus*, *Escherichia coli* i *Pseudomonas aeruginosa* (Dahham i in., 2015; Dickson i in., 2023). Dodatkowo (*E*)-*β*-kariofilen działa jako selektywny agonista receptorów kannabinoidowych CB2, co nadaje mu właściwości przeciwzapalne, pozbawione efektów psychoaktywnych typowych dla receptorów CB1. Mechanizm przeciwzapalny (*E*)-*β*-kariofilenu obejmuje m.in. hamowanie prozapalnych cytokin, takich jak TNF-α i IL-6 (Gertsch i in., 2008; Jha i in., 2021). Związek ten wpływa korzystnie także na metabolizm glukozy i lipidów, co ma pozytywny wpływ w przypadku cukrzycy i innych zaburzeń metabolicznych (Hashiesh i in., 2020). Kolejnym istotnym związkiem seskwiterpenowym jest *δ*-kadinen, charakteryzujący się działaniem przeciwbakteryjnym i przeciwzapalnym. Jego aktywność antybakteryjna wynika z destabilizacji błon komórkowych bakterii prowadzącej do ich śmierci. W badaniach wykazano, że *δ*-kadinen działa w szerokim spektrum bakterii, w tym na *Streptococcus pneumoniae* (Pérez-López i in., 2011; González i in., 2012), wykazuje również właściwości przeciwgrzybicze (Kundu i in., 2013). Podobnie jak inne składniki olejków eterycznych kocanki *δ*-kadinen działa przeciwzapalnie poprzez modulowanie poziomu cytokin i mediatorów zapalnych (Costa, i in., 2022). Kolejne ważne składniki olejków eterycznych z kocanek z grupy monoterpenów to *α*- i *β*-pineny oraz limonen, które charakteryzują się właściwościami antybakteryjnymi i przeciwzapalnymi. Badania wskazują, że *α*-pinen jest szczególnie skuteczny wobec bakterii *S. aureus* i *E. coli,* a jego działanie wzmacniane jest w połączeniu z innymi substancjami o aktywności antybakteryjnej. Zarówno *α*-pinen, jak i *β*-pinen mają zdolność do redukcji reaktywnych form tlenu (ROS), co może przyczyniać się do zmniejszenia stresu oksydacyjnego na poziomie komórkowym i wskazywać na ich potencjał jako naturalnych antyoksydantów (Salehi i in., 2019). Limonen natomiast wykazuje

potencjalne właściwości przeciwnowotworowe, m.in. poprzez indukcję apoptozy w komórkach nowotworowych oraz hamowanie ich proliferacji (Ribeiro i in., 2023; Lin i in., 2024).

Do analizy kwiatostanów oraz liści i łodyg *H. arenarium* wykorzystano metodę HS-SPME, mającą na celu efektywną izolację i charakterystykę składników frakcji lotnych znad surowca, odpowiadających za właściwości zapachowe tej rośliny. Metoda ta, ceniona za krótki czas ekstrakcji, niski koszt analizy oraz możliwość wielokrotnego wykorzystania włókien SPME (Pawliszyn, 1997), pozwoliła na uchwycenie szerokiego spektrum komponentów zapachowych, szczególnie tych wysoce lotnych, z różnych części badanej rośliny. Wyższa zawartość monoterpenów zaobserwowana w lotnych frakcjach z części zielonych rośliny może wskazywać na ich szczególną rolę w mechanizmach obronnych rośliny przed patogenami (Hammerbacher i in., 2019). Natomiast przewaga węglowodorów seskwiterpenów w olejkach eterycznych, uzyskanych metodą destylacji z parą wodną, podkreśla ich potencjalne znaczenie jako składników bioaktywnych znajdujących zastosowanie w aromaterapii i kosmetologii (Koyama i Heinbockel, 2020).

Na podstawie przeprowadzonych analiz składu chemicznego olejków eterycznych i frakcji lotnych *H. arenarium* można wnioskować, że roślina ta wykazuje dość wysoką stabilność w zakresie jakości i liczby kluczowych składników zapachowych, niezależnie od miejsca zbioru materiału roślinnego. Zróżnicowanie kolorystyczne olejków wskazuje jednak na subtelne różnice w składzie chemicznym zależnie od lokalizacji, co może sugerować wpływ czynników środowiskowych na biosyntezę niektórych związków. Niebieską barwę olejku pozyskanego z Podlasia determinuje chamazulen, zidentyfikowany na poziomie do 1,4%. Analiza frakcji lotnych metodą HS-SPME również dostarczyła precyzyjnych danych na temat profilu wysoce lotnych związków tej rośliny. Znacząca obecność (*E*)-*β*-kariofilenu, kluczowego seskwiterpenu, ściśle wiąże się z działaniem przeciwzapalnym i przeciwbakteryjnym kocanek, co wspiera ich tradycyjne zastosowania w łagodzeniu stanów zapalnych oraz ochronie przed infekcjami. Wysoka zawartość monoterpenów, takich jak *α*-pinen i *β*-pinen oraz limonen, w olejkach lub frakcjach lotnych także ma istotne znaczenie w terapeutycznym zastosowaniu kocanek, ponieważ ich właściwości przeciwzapalne korespondują z tradycyjnym wykorzystaniem rośliny w leczeniu schorzeń wątroby, dróg żółciowych oraz układu pokarmowego, gdzie przewlekłe stany zapalne często stanowią kluczowy problem. Ponadto monoterpeny wzbogacają przyjemny, świeży aromat kocanek, co zwiększa ich zastosowanie w aromaterapii. W rezultacie składniki olejkowe w postaci seskwiterpenów i monoterpenów nie tylko podkreślają wszechstronność działania *H. arenarium*, ale także wzbogacają ich zastosowanie w nowoczesnym ziołolecznictwie i kosmetologii.

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## Bibliografia

Agatonovic-Kustrin S., Gegechkori V., Kobakhidze T., Morton D. 2023. Solid-Phase Microextraction Techniques and Application in Food and Horticultural Crops. *Molecules* 28, nr art. 6880. DOI: https://doi.org/10.3390/molecules28196880.

Akaberi M., Sahebkar A., Azizi N., Emami S.A. 2019. Everlasting flowers: Phytochemistry and pharmacology of the genus *Helichrysum*. *Industrial Crops and Products* 138, nr art. 111471. DOI: 10.1016/j.indcrop.2019.111471.

Babushok V.I., Linstrom P.J., Zenkevich I.G. 2011. Retention Indices for Frequently Reported Compounds of Plant Essential Oils. *Journal of Physical and Chemical Reference Data* 40, nr art. 043101. DOI: 10.1063/1.3653552.

Costa C.C., Leão E.M., Campos C.S.B., Silva B.F.L., Lucas E.M.F., Machado A.M.R. 2022. Variation on the Terpene Profile of *Ocimum basilicum* Leaf Tea Caused by Leaves Storage Conditions and Preparation Methods. *Research, Society and Development* 11, nr art. e244111537201. DOI: 10.33448/rsd-v11i15.37201.

Dahham S.S., Tabana Y.M., Iqbal M.A., Ahamed M.B.K., Ezzat M.O., Majid A.S.A., Majid A.M.S.A. 2015. The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of *Aquilaria crassna*. *Molecules* 20, str. 11808-11829. DOI: 10.3390/molecules200711808.

Dănăilă-Guidea S.M., Eremia M.C., Dinu L.D., Miu D.M. 2022. *Helichrysum arenarium*: From Cultivation to Application. *Applied Sciences* 12, nr art. 10241. DOI: 10.3390/app122010241.

Dickson K., Scott C., White H., Zhou J., Kelly M., Lehmann C. 2023. Antibacterial and Analgesic Properties of Beta-Caryophyllene in a Murine Urinary Tract Infection Model. *Molecules* 28, nr art. 4144. DOI: 10.3390/molecules28104144.

European Medicines Agency (EMA). 2016. European Union Herbal Monograph on *Helichrysum arenarium* (L.) *Moench*, flos. EMA/HMPC/41108/2015. Dostępne online: www.ema.europa.eu/en/documents/herbal-monograph/final-europeanunion-herbal-monograph-helichrysum-arenarium-l-moench-flos_en.pdf (dostęp: 08.12.2024).

Farmakopea Polska (FP VI). 2002. Urząd Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Polskie Towarzystwo Farmaceutyczne, wyd. VI, Warszawa, str. 151.

Farmakopea Polska (FP XIII). 2023. Urząd Rejestracji Produktów Leczniczych, Wyrobów Medycznych i Produktów Biobójczych, Polskie Towarzystwo Farmaceutyczne, wyd. XIII, Warszawa, str. 4836–4837.

Gertsch J., Leonti M., Raduner S., Racz I., Chen J.-Z., Xie X.-Q., Altmann K.-H., Karsak M., Zimmer A. 2008. Beta-Caryophyllene is a Dietary Cannabinoid. *Proceedings of the National Academy of Sciences of the United States of America* 105(26), str. 9099–9104. DOI: 10.1073/pnas.0803601105.

González A.M., Tracanna M.I., Amani S.M., Schuff C., Poch M.J., Bach H., Catalán C.A.N. 2012. Chemical Composition, Antimicrobial and Antioxidant Properties of the Volatile Oil and Methanol Extract of *Xenophyllum poposum*. *Natural Product Communications* 7(12), str. 1663–1666. DOI: 10.1177/1934578X1200701230.

Hammerbacher A., Coutinho T.A., Gershenzon J. 2019. Roles of plant volatiles in defence against microbial pathogens and microbial exploitation of volatiles. *Plant, Cell & Environment* 42, str. 2827–2843. DOI: 10.1111/pce.13602.

Hashiesh H.M., Meeran M.F.N., Sharma C., Sadek B., Al Kaabi J., Ojha S.K. 2020. Therapeutic Potential of β-Caryophyllene: A Dietary Cannabinoid in Diabetes and Associated Complications. *Nutrients* 12, nr art. 2963. DOI: 10.3390/nu12102963.

Jha N.K., Sharma C., Hashiesh H.M., Arunachalam S., Meeran M.F.N., Javed H., Patil C.R., Goyal S.N., Ojha S. 2021. β-Caryophyllene, A Natural Dietary CB2 Receptor Selective Cannabinoid, Can Be a Candidate to Target the Trinity of Infection, Immunity, and Inflammation in COVID-19. *Frontiers in Pharmacology* 12, nr art. 590201. DOI: 10.3389/fphar.2021.590201.

Judžentienė A., Butkienė R. 2006. Chemical composition of the essential oils of wild *Helichrysum arenarium* (L.) with differently colored inflorescences from Eastern Lithuania. *Journal of Essential Oil Research* 18, str. 80–83. DOI: 10.1080/10412905.2006.9699391.

Koyama S., Heinbockel T. 2020. The Effects of Essential Oils and Terpenes in Relation to Their Routes of Intake and Application. *International Journal of Molecular Sciences* 21(5), str. 1558. DOI: 10.3390/ijms21051558.

Kundu A., Saha S., Walia S., Shakil N., Kumar J., Annapurna K. 2013. Cadinene Sesquiterpenes from *Eupatorium adenophorum* and Their Antifungal Activity. *Journal of Environmental Science and Health*, Part B 48, str. 516–522. DOI: 10.1080/03601234.2013.761921.

Lamer-Zarawska E., Kowal-Gierczak B., Niedworok J. (red.). 2007. *Fitoterapia i leki roślinne*. Wydanie 1. Wydawnictwo Lekarskie PZWL, Warszawa, str. 355–356.

Lin H., Li Z., Sun Y., Zhang Y., Wang S., Zhang Q., Cai T., Xiang W., Zeng C., Tang J. 2024. D-Limonene: Promising and Sustainable Natural Bioactive Compound. *Applied Sciences* 14, nr art. 4605. DOI: 10.3390/app14114605.

Liu X., Jing X., Li G. 2019. A process to acquire essential oil by distillation concatenated liquid-liquid extraction and flavonoids by solid-liquid extraction simultaneously from *Helichrysum arenarium* (L.) Moench inflorescences under ionic liquid microwave mediated. *Separation and Purification Technology* 209, str. 164–174. DOI: 10.1016/j.seppur.2018.07.028.

Pawliszyn J. 1997. *Solid Phase Microextration – Theory and Practice*, Wiley-VCH, New York.

Pérez-López A., Torres Cirio A., Rivas-Galindo V.M., Salazar Aranda R., Waksman de Torres N. 2011. Activity against *Streptococcus pneumoniae* of the Essential Oil and d-Cadinene Isolated from *Schinus molle* Fruit. *Journal of Essential Oil Research* 23, nr art. 25. DOI: 10.1080/10412905.2011.9700477.

Pljevljakušić D., Bigović D., Janković T., Jelačić S., Šavikin K. 2018. Sandy Everlasting (*Helichrysum arenarium* (L.) Moench): Botanical, Chemical and Biological Properties. *Frontiers in Plant Science* 9, nr art. 1123. DOI: 10.3389/fpls.2018.01123.

Rančić A., Soković M., Vukojević J., Simić A., Marin P., Duletić-Laušević S., Djoković D. 2005. Chemical Composition and Antimicrobial Activities of Essential Oils of *Myrrhis odorata* (L.) Scop, *Hypericum perforatum* L and *Helichrysum arenarium* (L.) Moench *Journal of Essential Oil Research* 17(3), str. 341–345. DOI: 10.1080/10412905.2005.9698925.

Rassem H.H., Nour A.H., Yunus R.M. 2016. Techniques for Extraction of Essential Oils from Plants: A Review. *Australian Journal of Basic and Applied Sciences* 10, str. 117–127.

Reidel R.V.B., Cioni P.L., Ruffoni B., Cervelli C., Pistelli L. 2017. Aroma Profile and Essential Oil Composition of *Helichrysum* species. *Natural Product Communications* 12(9), str. 1507–1512. DOI: 10.1177/1934578X1701200931.

Ribeiro A.D., Cardoso M.N.A., Freire J.C.P., Figueirêdo Jr E.C., Costa M.M.A., Silva P.G., Gomes D.Q.C., Brito E.M.M., Pereira J.V. 2023. Antimicrobial Activity of Limonene: Integrative Review. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas* 22(5), str. 581–593. DOI: 10.37360/blacpma.23.22.5.42.

Salehi B., Upadhyay S., Erdogan Orhan I., Jugran A.K., Jayaweera S.L.D., Dias D.A., Sharopov F., Martins N., Baghalpour N., Cho W.C., Taheri Y., Sharifi-Rad J. 2019. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. *Biomolecules* 9, nr art. 738. DOI: 10.3390/biom9110738.

Sawilska A.K. 2015. *Biologiczne i ekologiczne zużycie introdukcji kocanek piaskowych Helichrysum arearium (L.) Moench do uprawy polowej*. W: Załuski T., Krasicka-Korczyńska E., Ratyńska H., Sawilska A.K. (red.), *Cenne składniki flory i roślinności na obszarze Pomorza i Kujaw*. Wydawnictwo Polskiego Towarzystwa Botanicznego, Bydgoszcz, str. 5–20.

Tığlı Kaytanlıoğlu E.H., Özderin S., Fakir H., Gümüşay E. 2021. Determination of Volatile Components of *Helichrysum arenarium* subsp. aucheri Naturally Distributed in Two Different Regions. *European Journal of Science and Technology* 25, str. 152–158. DOI: 10.31590/ejosat.887605.

Zheljazkov V.D., Semerdjieva I., Yankova-Tsvetkova E., Astatkie T., Stanev S., Dincheva I., Kačániová M. 2022. Chemical Profile and Antimicrobial Activity of the Essential Oils of *Helichrysum arenarium* (L.) Moench. and *Helichrysum italicum* (Roth.) G. Don. *Plants* 11(7), nr art. 951. DOI: 10.3390/plants11070951.

UMEDICAL REPORTS

# POZIOM ODCZUWANEGO STRESU U PACJENTÓW Z SUCHOŚCIĄ JAMY USTNEJ

## LEVEL OF PERCEIVED STRESS IN PATIENTS WITH XEROSTOMIA

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**Streszczenie:** Pacjenci zgłaszający się do gabinetu dentystycznego coraz częściej skarżą się na suchość w obrębie jamy ustnej. Istnieje wiele powodów zmniejszonego wydzielania śliny. Najczęściej problem ten jest związany z wiekiem oraz przewlekłym stosowaniem leków kserostomiogenicznych. Odczuwanie suchości oraz dyskomfortu może mieć znaczenie dla nasilenia odczuwanego stresu i radzenia sobie z nim. Celem badania była ocena natężenia stresu w grupie pacjentów zgłaszających suchość w obrębie jamy ustnej w porównaniu z osobami nieodczuwającymi suchości. **Materiały i metody.** Badaniem objęto 90 pacjentów: 60 osób zgłaszało suchość w obrębie jamy ustnej, 30 pacjentów było bez takich dolegliwości. Każdy z uczestników podpisał formularz zgody na udział w badaniu. U wszystkich badanych dokonano pomiaru wydzielania śliny niestymulowanej w czasie t = 5 min, badania jamy ustnej oraz zebrano odpowiedzi w teście natężenia na stres PSS-10. **Wyniki.** Uzyskano istotne statystycznie różnice w wynikach testu PSS-10 oraz pomiaru wydzielania śliny niestymulowanej pomiędzy badanymi grupami. Osoby z suchością jamy ustnej prezentowały wyższe parametry stresu. **Wnioski.** w przypadku osób z suchością jamy ustnej, z uwagi na wyższe parametry stresu, wymagane jest rozważenie włączenia do leczenia procedur kontroli stresu.

**Słowa kluczowe:** kserostomia, stres, starzenie się, jakość życia

**Abstract:** Patients presenting to the dental surgery are increasingly complaining of oral dryness. There are several reasons for the decreased saliva secretion. Most commonly, the problem is related to age and chronic use of xerostomiogenic drugs. The perception of dryness and discomfort may be relevant to the severity of perceived stress management. The aim of this study was to assess the severity of stress in a group of patients reporting oral dryness compared with those not perceiving dryness. **Material and methods.** 90 patients were included in the study: 60 of them reported dry mouth, 30 patients were without such complaints. Each participant signed a consent form to take part in the study. In all patients, oral examination was performed and unstimulated saliva secretion at t=5min and the responses to the PSS-10 stress intensity test were measured. **Results.** Statistically significant differences were obtained in the results of the PSS-10 test and the measurement of unstimulated saliva secretion between the groups with and without xerostomy. Those with oral dryness presented higher stress parameters. **Conclusions.** People with xerostomia, due to higher stress parameters, need to consider including stress control procedures in their treatment.

**Keywords:** xerostomia, stress, aging, quality of life

**Wykaz skrótów**

**OHRQoL** – jakość życia związana ze stanem jamy ustnej (ang. *oral health-related quality of life*)
**PSS-10** – Skala Odczuwanego Stresu (ang. The Perceived Stress Scale)
**SXI-5** – sumaryczny wskaźnik kserostomii (ang. *summated xerostomia inventory*)

## 1. Wprowadzenie

Współcześnie zdrowie psychiczne i jego powiązania ze stanem fizycznym oraz dobrostanem społecznym są coraz częściej przedmiotem zainteresowania naukowców. Dane dotyczące zmian w populacji – w Polsce i w Europie – wskazują na postępujące starzenie się społeczeństwa (United Nations, 2022; GUS, 2014). Za grupę najszybciej rosnącą liczebnie uważa się osoby powyżej 65. roku życia.

Obserwowane w jamie ustnej zmiany związane z wiekiem dotyczą między innymi zmniejszenia wydzielania śliny (Liu i in., 2012). Obniżenie tego parametru ma niekorzystny wpływ na wiele aspektów funkcjonowania jednostki. Znacząco upośledza codzienne czynności, takie jak: przeżuwanie, połykanie czy mówienie oraz negatywnie wpływa na stan błony śluzowej jamy ustnej oraz zębów (Herrmann i in., 2017; Niklander i in., 2017). Uważa się, że kserostomia może dotyczyć 10–30% społeczeństwa, przeważnie osób starszych (Fornari i in., 2021; Pichór i in., 2008).

Pod względem ilości wydzielanej śliny rozróżnia się kserostomię prawdziwą (*xerostomia vera, primaria*) oraz kserostomię rzekomą (*xerostomia spuria, symptomatica*). Kserostomię prawdziwą, w której odczuwanie suchości wynika ze zmniejszonego wydzielania śliny (poniżej 0,1 ml/min w pomiarze śliny niestymulowanej) można podzielić na dwa typy:

- typ I – nie obserwuje się tu zmian klinicznych w obrębie błony śluzowej jamy ustnej,
- typ II – wykazuje obecność zmian zanikowych oraz zmian wewnątrz gruczołów ślinowych.

Kserostomia rzekoma jest diagnozowana u pacjentów zgłaszających dolegliwości związane z suchością, lecz prezentujących prawidłowe parametry wydzielania śliny. Odczucie suchości wynika głównie ze stosowania leków kserostomiogenicznych, a także z powodu cukrzycy, anemii, bulimii, depresji, palenia papierosów, alkoholizmu oraz narkomanii (Tanasiewicz i in., 2016). Możliwym czynnikiem wyzwalającym i podtrzymującym może być również stres (Gholami i in., 2017; Bulthuis i in., 2018; Atif i in., 2020). Dodatkowo permanentne uczucie suchości w jamie ustnej może ten stres nasilać.

## 2. Materiały i metody

Badaniem objęto grupę 90 osób (66 kobiet oraz 24 mężczyzn) w wieku 38–90 lat zgłaszających się do Poradni Patologii Jamy Ustnej Instytutu Stomatologii w Łodzi w latach 2019–2022 w celu sanacji jamy ustnej. Badanie uzyskało pozytywną opinię Komisji Bioetycznej Uniwersytetu Medycznego w Łodzi (nr RNN/100/19/KE). Wszystkie osoby biorące udział w badaniu zapoznały się z informacją o nim oraz wyraziły pisemną zgodę. Badanie było proste i nieinwazyjne.

U każdego z uczestników przeprowadzono badanie stomatologiczne, dokonano oceny stanu błony śluzowej jamy ustnej, potrzeb leczniczych, a także pomiaru ilości wydzielanej śliny niestymulowanej w czasie t = 5 minut (metoda odpluwania śliny do jednorazowego kubeczka w warunkach komfortowych i pozycji siedzącej oraz ocena jej ilości po czasie 5 minut przy pomocy mechanicznej pipety z jednorazowymi końcówkami) oraz zebrano odpowiedzi na pytania w kwestionariuszu PSS-10. Kwestionariusz ten jest polską interpretacją Skali Odczuwanego Stresu (*Perceived Stress Scale*) autorstwa Sheldona Cohena, Toma Kamarcka i Robin J. Mermelstein (adaptacji podjęli się Zygfryd Juczyńskio i Nina Ogińska-Bulik). Kwestionariusz PSS-10 składa się z dziesięciu pytań, pomocnych przy ocenie poziomu odczuwanego stresu psychicznego w miesiącu poprzedzającym badanie. Pacjenci odpowiadają na pytania, posługując się pięciostopniową skalą Likerta: jeżeli w danym czasie pacjent nigdy nie spotkał się ze wspomnianą sytuacją, wybiera 0, prawie nigdy – 1, czasem – 2, dość często – 3, a bardzo często – 4. W badaniu znajdują się pytania odnoszące się zarówno do pozytywnych, jak i negatywnych doświadczeń (odpowiednio 4 i 6 pytań). Wynik dla każdego pytania wynosi od 0 do 4 punktów, a ogólny wynik skali, będący sumą wszystkich punktów, od 0 do 40. Im wyższy wynik, tym większe nasilenie odczuwanego stresu. Niskiemu poziomowi odczuwanego stresu odpowiadają wyniki w przedziale od 0 do 13 punktów, średniemu poziomowi – punkty od 14 do 19, natomiast wysokiemu poziomowi – wynik od 20 do 40 punktów (Juczyński i Ogińska-Bulik., 2009). Ze względu na niewielką liczbę niezbyt skomplikowanych pytań skala jest łatwa i mało absorbująca (na wypełnienie kwestionariusza pacjent otrzymuje maksymal-

nie pięć minut), z tego względu jest bardzo przydatna i łatwa do zastosowania w codziennej praktyce lekarzy i lekarzy dentystów.

Do analizy statystycznej użyto programu Statistica 13.3 (TIBCO Software Inc.). Za próg istotności statystycznej przyjęto $\alpha = 0,05$. Do oceny normalności rozkładu zmiennych użyto testu Shapiro–Wilka. Różnice pomiędzy grupami w wielkości badanych parametrów oceniano przy użyciu testu U Manna–Whitneya.

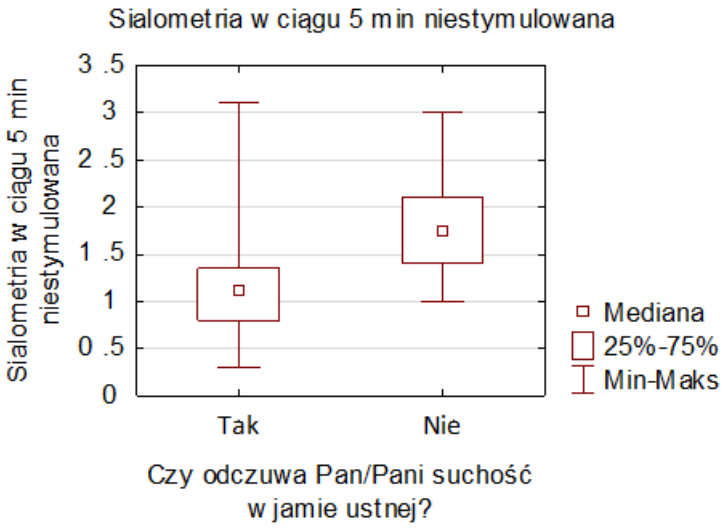
## 3. Wyniki

Spośród całej grupy 60 pacjentów (45 kobiet oraz 15 mężczyzn) w wieku 38–90 lat zgłaszało suchość w obrębie jamy ustnej, natomiast 30 osób (21 kobiet oraz 9 mężczyzn) w wieku 54–82 lat nie zgłaszało suchości. Żadna z badanych zmiennych nie wykazywała rozkładu normalnego. Średni wiek w badanej grupie wyniósł 67,88 lat (Me = 68; Min = 38; Max = 90; SD = 11,27). Mężczyźni (Me = 65,5; Min = 45; Max = 80; SD = 10,66) biorący udział w badaniu byli istotne statystycznie młodsi ($p = 0,0294$) w porównaniu z kobietami (Me = 70; Min = 38; Max = 90; SD = 11,14). Nie wykazano istotnych statystycznie różnic w wieku między grupą z suchością jamy ustnej i bez suchości.

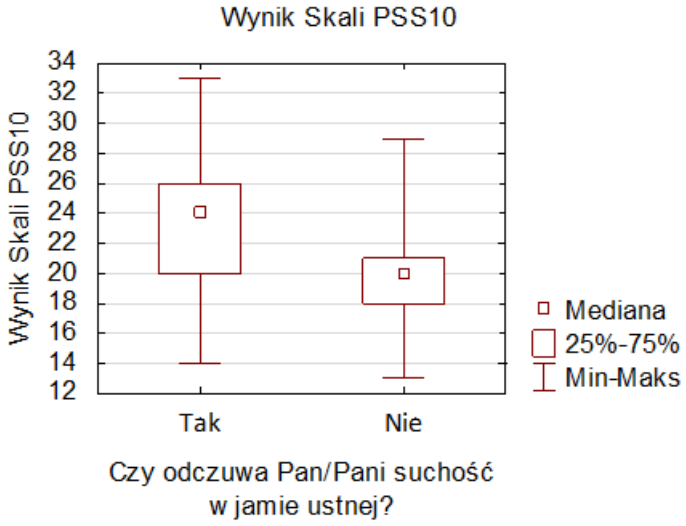
Analiza statystyczna wykazała istotne statystycznie różnice w wynikach sialometrii niestymulowanej mierzonej w czasie t = 5 minut (ml/5min) ($p < 0,0001$) pomiędzy grupą z suchością jamy ustnej (M = 1,16; Me = 1,10; Min = 0,30; Max = 3,10; SD = 0,49) a grupą badanych tej dolegliwości niezgłaszających (M = 1,75; Me = 1,75; Min = 1,00; Max = 3,00; SD = 0,45) (Tabela 1, Rycina 1).

Wykazano także różnice w przepływie śliny mierzonej w ml/min ($p < 0,0001$). Średnia wartość tego parametru w grupie z suchością jamy ustnej wyniosła 0,23 ml/min (Me = 0,22; Min = 0,06; Max = 0,62; SD = 0,10), a w grupie bez suchości 0,35 ml/min (Me = 0,35; Min = 0,20; Max = 0,60; SD = 0,09) (Tabela 1).

Analiza różnic skali odczuwania stresu (PSS-10) wykazała, że pacjenci z suchością jamy ustnej uzyskiwali istotne statystycznie wyższe wyniki w porównaniu z pacjentami bez suchości ($p = 0,0011$) (Tabela 2, Rycina 2). Średni wynik skali u pacjentów odczuwających suchość w jamie ustnej wyniósł 23,18 (Me = 24; Min = 14; Max = 33; SD = 4,13), a w grupie badanych nieodczuwających suchości 20,17 (Me = 20; Min = 13; Max = 29; SD = 3,39). Wykazano także istotne statystycznie różnice pomiędzy grupami w kontekście odpowiedzi udzielanych na pytania: „Jak często w ciągu ostatniego miesiące byłeś(-łaś) zdenerwowany(-na), ponieważ zdarzyło się coś niespodziewanego?" ($p = 0,0257$); „Jak często w ciągu ostatniego miesiąca czułeś(-łaś), że ważne sprawy w twoim życiu wymykają Ci się spod kontroli?" ($p = 0,0101$); „Jak często w ciągu ostatniego miesiąca odczuwałeś(-łaś) zdenerwowanie i napięcie?" ($p = 0,0020$); „Jak często w ciągu ostatniego miesiąca czułeś(-łaś), że wszystko Ci wychodzi?" ($p = 0,0256$), „Jak często w ciągu ostatniego miesiąca złościłeś(-łaś) się, ponieważ nie miałeś(-łaś) wpływu na to, co się zdarzyło?" ($p = 0,0063$) oraz „Jak często w ciągu ostatniego miesiąca czułeś(-łaś), że nie możesz przezwyciężyć narastających trudności?"($p = 0,0003$).




**Rycina 1.** Odczucie suchości w jamie ustnej względem ilości wydzielanej śliny.




**Rycina 2.** Wyniki testu PSS-10 względem odczuwania suchości w jamie ustnej.

**Tabela 1.** Wyniki dla sialometrii niestymulowanej w czasie t=5min.

| | | **Średnia (M)** | **Mediana (Me)** | **Minimum (Min)** | **Maksimum (Max)** | **Odchylenie standardowe (SD)** |
|---|---|---|---|---|---|---|
| Sialometria w ciągu 5 minut niestymulowana (ml/5 min) | Ogółem | 1,36 | 1,30 | 0,30 | 3,10 | 0,55 |
| | Odczuwana suchość | 1,16 | 1,10 | 0,30 | 3,10 | 0,49 |
| | Brak odczucia suchości | 1,75 | 1,75 | 1,00 | 3,00 | 0,45 |
| Wydzielanie minutowe (ml/min) | Ogółem | 0,27 | 0,26 | 0,06 | 0,62 | 0,11 |
| | Odczuwana suchość | 0,23 | 0,22 | 0,06 | 0,62 | 0,10 |
| | Brak odczucia suchości | 0,35 | 0,35 | 0,20 | 0,60 | 0,09 |

**Tabela 2.** Wyniki w teście PSS-10

| | **Średnia (M)** | **Mediana (Me)** | **Minimum (Min)** | **Maksimum (Max)** | **Odchylenie standardowe (SD)** |
|---|---|---|---|---|---|
| Ogółem | 22,18 | 22,00 | 13,00 | 33,00 | 4,13 |
| Odczuwana suchość | 23,18 | 24,00 | 14,00 | 33,00 | 4,13 |
| Brak odczucia suchości | 20,17 | 20,00 | 13,00 | 29,00 | 3,39 |

## 4. Dyskusja

Do tej pory powstało stosunkowo niewiele badań dotyczących odczuwania stresu u pacjentów zgłaszających suchość w obrębie jamy ustnej. Atif i in. (2020) przeprowadzili badanie z udziałem 72 studentów obejmujące pomiar ilości śliny niestymulowanej oraz ocenę odczuwania stresu przy pomocy testu PSS-10. Średnia wydzielania śliny niestymulowanej w czasie t = 5 min wynosiła 1,3 ml, średni przepływ śliny 0,26 ml/min. Uczestnicy badania z wyższym odczuwaniem stresu (wynik PSS-10 > 23) prezentowali niższe wartości wydzielania śliny w porównaniu z uczestnikami

odczuwającymi mniejsze natężenie stresu. Wykazano umiarkowaną ujemną korelację pomiędzy przepływem śliny a odczuwaniem stresu ($r$ = −0,259, $p$ = 0,028).

Badania Botelho i in. (2020) na grupie 592 osób powyżej 65. roku życia (śr. wieku 72,6 lat, SD = 6,4) w Portugalii wykazały istotną statystycznie korelację ($p < 0,001$) pomiędzy odczuwanym stresem (PSS-10), suchością w jamie ustnej ocenianą przy użyciu ankiety subiektywnego odczuwania suchości w jamie ustnej (ang. Summated Xerostomia Inventory, SXI-5) a pogorszeniem jakości życia związanym z jamą ustną (ang. *oral health related quality of life*, OHRQoL). Średnia wartość testu PSS-10 uzyskanego wśród uczestników badania wynosiła 15,1 (SD = 8,0). Średni wynik w teście SXI-5 wynosił 6,7 (SD = 1,9) (Botelho i in., 2020).

Z kolei badania przeprowadzone w Malezji przez Muzhaffar i in. (2023) na 245 studentach na podstawie Summated Xerostomia Inventory (SXI) oraz testu PSS-10 wykazały istotną statystycznie ($p < 0,01$) zależność wskazującą na zwiększone odczuwanie stresu u uczestników zgłaszających większą uciążliwość suchości w jamie ustnej.

Wyniki uzyskane w badaniach własnych, prowadzonych w Polsce, są zgodne z obserwacjami innych badaczy. Wskazują one na zwiększone odczuwanie stresu u pacjentów zgłaszających suchość w obrębie jamy ustnej. Średnie wydzielanie śliny niestymulowanej w czasie t = 5min u osób zgłaszających suchość w jamie ustnej wynosiło 1,16 ml, a średni wynik testu PSS-10 wyniósł 23,18. W grupie osób niezgłaszających suchości wyniki wynosiły odpowiednio 1,75 ml i 20,17. Różnice pomiędzy grupami były istotne statystycznie zarówno pod względem ilości wydzielanej śliny niestymulowanej ($p < 0,0001$), jak i wyników w teście PSS-10 ($p = 0,0011$). Z racji przewlekłego charakteru kserostomii zwiększone odczuwanie stresu u pacjentów z suchością w jamie ustnej może prowadzić z czasem do pogorszenia stanu psychicznego oraz do rozwoju depresji. Stan zdrowia psychicznego pacjentów oraz ich umiejętność radzenia sobie ze stresem wydają się być marginalizowane w codziennej praktyce stomatologicznej. W opiece stomatologicznej próbuje się dołączyć metody kontroli stresu do standardowego postępowania diagnostyczno-terapeutycznego.

Badania przeprowadzone przez Szymczak-Paluch i Kłoska (2023) sugerują większą skuteczność w leczeniu liszaja płaskiego błony śluzowej jamy ustnej, gdy standardowa kuracja jest połączona z aktywnymi metodami kontroli stresu psychicznego. Stosowanie metod redukcji stresu zmniejszało również odczucie bólu w obrębie błony śluzowej, przyczyniając się do lepszego stanu ogólnego pacjentów. Tym bardziej zasadne wydaje się wprowadzenie oceny parametrów stresu w diagnostyce, a docelowo – wdrożenie metod radzenia sobie ze stresem w trakcie leczenia kserostomii. Dla potwierdzenia uzyskanych danych wskazane byłoby przeprowadzenie dalszych, długofalowych badań na większej grupie osób.

## 5. Wnioski

Pomimo ograniczeń badania można stwierdzić, iż osoby z suchością prezentują wyższe parametry stresu. Powinno to zwiększyć uwagę lekarzy i lekarzy dentystów na problemy, jakim są odczuwanie suchości w jamie ustnej oraz zdrowie psychiczne pacjentów. Należałoby rozważyć interdyscyplinarną współpracę z psychologami i fizjoterapeutami oraz włączenie procedur kontroli stresu podczas leczenia pacjentów zgłaszających się z objawami kserostomii.

## Bibliografia


Atif S., Syed S.A., Sherazi U.R., Rana S. 2020. Determining the relationship among stress, xerostomia, salivary flow rate, and the quality of life of undergraduate dental students. *Journal of Taibah University Medical Sciences* 16(1), str. 9–15. DOI: 10.1016/j.jtumed.2020.10.019.

Botelho J., Machado V., Proenca L., Oliveira M.J., Cavacas M.A., Amaro L., Águas A., Mendes J.J. 2020. Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: Findings from a cross-sectional survey. *BMC Oral Health* 20(1), nr art. 199. DOI: 10.1186/s12903-020-01183-7.

Bulthuis M.S., Jan Jager D.H., Brand H.S. 2018. Relationship among perceived stress, xerostomia, and salivary flow rate in patients visiting a saliva clinic. *Clinical Oral Investigations* 22(9), str. 3121–3127. DOI: 10.1007/s00784-018-2393-2.

Fornari C.B., Bergonci D., Stein C.B., Agostini B.A., Rigo L. 2021. Prevalence of xerostomia and its association with systemic diseases and medications in the elderly: A cross-sectional study. *Sao Paulo Medical Journal* 139(4), str. 380–387. DOI: 10.1590/1516-3180.2020.0616.R3.1902021.

Gholami N., Hosseini Sabzvari B., Razzaghi A., Salah S. 2017. Effect of stress, anxiety and depression on unstimulated salivary flow rate and xerostomia. *Journal of Dental Research, Dental Clinics, Dental Prospects* 11(4), str. 247–252. DOI: 10.15171/joddd.2017.043.

GUS. 2014. Prognoza ludności na lata 2014–2050. Dostępne online: https://stat.gov.pl/obszary-tematyczne/ludnosc/prognoza-ludnosci/prognoza-ludnosci-na-lata-2014-2050-opracowana-2014-r-,1,5.html (dostęp: 19.10.2023).

Herrmann G., Müller K., Behr M., Hahnel S. 2017. Xerostomia and its impact on oral health-related quality of life. *Zeitschrift für Gerontologie und Geriatrie* 50(2), str. 145–150. DOI: 10.1007/s00391-015-0968-y.

Juczyński Z., Ogińska-Bulik N. 2009. Skala Odczuwanego Stresu PSS-10. Warszawa: Pracownia Testów Psychologicznych Polskiego Towarzystw Psychologicznego.

Liu B., Dion M.R., Jurasic M.M., Gibson G., Jones J.A. 2012. Xerostomia and salivary hypofunction in vulnerable elders: Prevalence and etiology. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology* 114 (1), str. 52–60. DOI: 10.1016/j.oooo.2011.11.014.

Muzhaffar E., Berahim Z., Abdul Wahab N., Saddki N. 2023. Perceived stress, severity of xerostomia, and periodontal status in undergraduate dental students. *Journal of Dentistry Indonesia* 30(2), str. 121–127. DOI: 10.14693/jdi.v30i2.1500.

Niklander S., Veas L., Barrera C., Fuentes F., Chiappini G., Marshall M. 2017. Risk factors, hyposalivation and impact of xerostomia on oral health-related quality of life. *Brazilian Oral Research* 31, nr art. 14. DOI: 10.1590/1807-3107BOR-2017.vol31.0014.

Pichór A., Doboszyńska A. 2008. Suchość jamy ustnej – niedoceniany problem kliniczny. *Medycyna Paliatywna w Praktyce* 2(1), str. 26–28.

Szymczak-Paluch M., Kłosek S. 2023. Stress control as a method to reduce perceived pain in oral lichen planus. *Postępy Dermatologii i Alergologii* 40(2), str. 241–245. DOI: 10.5114/ada.2023.127641.

Tanasiewicz M., Hildebrandt T., Obersztyn I. 2016. Xerostomia of various etiologies: A review of the literature. *Advances in Clinical and Experimental Medicine* 25(1), str. 199–206. DOI: 10.17219/acem/29375.

United Nations. 2022. World Population Prospects. World population by age and sex: 2024. Dostępne online: https://population.un.org/wpp/Graphs/DemographicProfiles/Pyramid/900 (dostęp: 19.10.2023).

UMEDICAL REPORTS

# THE ROLE OF PROBIOTICS IN PERIODONTAL DISEASES

## ROLA PROBIOTYKÓW W CHOROBACH PRZYZĘBIA

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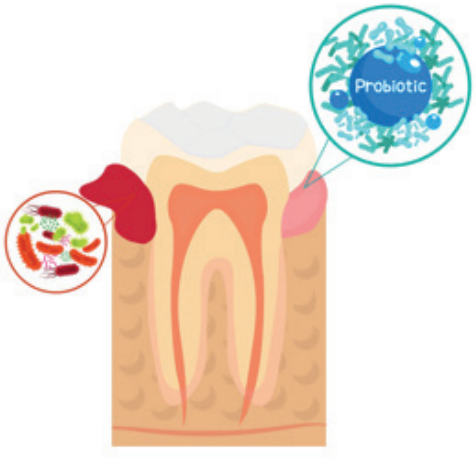

**Streszczenie:** Artykuł przedstawia alternatywną rolę probiotyków, prebiotyków i synbiotyków w leczeniu wieloczynnikowej choroby przyzębia, będącej coraz częstszym problemem osób w każdym wieku.

Probiotyki, spożywane w odpowiednich ilościach zapewniają pozytywny wpływ na nasze zdrowie w tym: całego układu pokarmowego, sercowo-naczyniowego oraz układu oddechowego imoczowego. Te komensalne mikroorganizmy zasiedlające naszą jamę ustną, wspomagają układ immunologiczny, wydzielają substancje bakteriobójcze i utrzymują homeostazę, stanowiąc alternatywną metodę leczenia chorób jamy ustnej w tym zapalenie przyzębia. Na szczególną uwagę zasługują naturalnie występujące komensalne bakterie jamy ustnej*: Lactobacillus species, Bifidobacterium* (LAB)*,* tak samo jak *Saccharomyces, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Bacillus, Escherichia* oraz gatunki o szerokim spektrum działania*: Lactiplantibacillus pentosus KCA1, Lactiplantibacillus plantarum WCFS1, Lacticaseibacillus rhamnosus GG* oraz *Limosilactobacillus reuteri AMBF471* wykorzystywane w terapii wspomagającej leczenie przyzębia.

Wykazujące synergizm działania synbiotyki, powstałe z połączenia probiotyków i prebiotyków, w ostatnim czasie zyskują coraz większą popularność. Badania wykazały ich znaczący wpływ na ograniczanie adhezji jednego z najczęściej występujących periopatogenów *Porphyromonas gingivalis.*

Ostateczne potwierdzenie korzystnego wpływu komensalnych bakterii oraz prebiotyków wymaga czasu i kontynuacji badań. Choroba ta niesie za sobą trudno odwracalne konsekwencje, które rozpoczynają się od zaburzenia homeostazy jamy ustnej, którą można powstrzymać na początkowym etapie zapalenia dziąseł. Z dotychczas przeprowadzonych badań, wynika ich pozytywny wpływ na hamowanie wzrostu i adhezji periopatogenów do błony śluzowej gospodarza, pobudzanie jego odpowiedzi immunologicznej, inhibicji procesu niszczenia kości wyrostka zębodołowego, polepszenia wskaźników periodontologicznych i ograniczenia pierwszego etapu chorób przyzębia - zapalenia dziąseł. Stanowią one obiecującą metodę we wspomaganiu leczenia przyzębia na każdym etapie jej występowania.

**Słowa kluczowe:** probiotyki, synbiotyki, zapalenie przyzębia, stomatologia, mikrobiota

**Abstract:** The article explores the alternative role of probiotics, prebiotics and synbiotics in the management of multifactorial periodontal diseases.

Supplemented in appropriate amounts, probiotics can positively impact overall health. These commensal microorganisms inhabit the oral cavity, support the immune system, release bactericidal substances, and maintain homeostasis, which makes them a potential alternative treatment for oral diseases, including gingivitis and periodontitis. Among the commensal bacteria naturally occupying the oral cavity, particularly noteworthy are *Lactobacillus* species, *Bifidobacterium* (LAB), as well as specific strains, such as *Lactiplantibacillus pentosus KCA1, Lactiplantibacillus plantarum WCFS1, Lacticaseibacillus rhamnosus GG*, and *Limosilactobacillus reuteri AMBF471.*

Despite promising findings, further research is necessary to definitively confirm the beneficial impact of commensal bacteria and prebiotics on periodontal health. Periodontal diseases, which begin with disturbances in oral homeostasis and early-stage gingivitis, can lead to irreversible consequences. Nonetheless, an appropriate microbiological support may prevent progression in the initial stages. Current studies indicate that probiotics and synbiotics can inhibit the growth of periopathogens on the host's mucosal surface, stimulate the host immune response, improve periodontal indicators and control the initial stages of periodontal diseases - gingivitis. Thus, they represent a promising adjunctive approach for managing gingivitis and periodontitis at various stages of their progression.

**Keywords**: probiotics, synbiotics, periodontitis, dentistry, microbiota

## Introduction

The red complex, consisting of the bacteria Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, along with the pathogen Aggregatibacter actinomycetemcomitans, is considered the main bacterial agent of periodontal disease in periodontology (Shirbhate et al., 2023). This multifactorial disease affects from 20% to 50% of the global population (Nazir, 2017). Apart from the presence of this complex, the disease often originates from inadequate oral hygiene, leading to accumulation of supragingival plaque and subsequent local inflammation. If oral hygiene is not improved, this inflammation progresses to marginal gingivitis, the initial, reversible stage of periodontitis (Rathee and Jain, 2023). Further neglect of hygiene practices can result in the destruction of periodontal tissues, tooth loss, and resorption of the alveolar bone, conditions collectively referred to as periodontitis (Hardan et al., 2022; Shirbhate et al., 2023).

No specific medication can directly treat periodontal disease; however, dental practices include procedures that slow its progression. The primary intervention is scaling and root planing (SRP), which may be supplemented by systemic or local antibiotics. Yet, due to widespread antibiotic resistance, probiotics, as commensal microorganisms, are increasingly used to support mechanical therapy (Hardan et al., 2022; Kaźmierczyk-Winciorek et al., 2021; Nguyen et al., 2021).

Probiotics are live, commensal organisms that not only benefit oral health but also support the intestines, respiratory system, cardiovascular system, and the female urinary tract (Chen et al., 2023; Haas et al., 2021; Hardan et al., 2022; Homayouni Rad et al., 2023; Inchingolo et al., 2023; Kaźmierczyk-Winciorek et al., 2021; Lee et al., 2021; Nguyen et al., 2021; Rebelo et al., 2023; Teles et al., 2022). Their mechanism of action involves direct and indirect competition with pathogenic microorganisms, initiating immune processes at sites affected by microbes disadvantageous to the host (Inchingolo et al., 2023). Additionally, probiotics stimulate both cellular and systemic immune responses (Shirbhate et al., 2023). Due to the short transit time within the oral cavity, one measure in the evaluation and classification of probiotic strains is their capacity to adhere to the host's mucosal membrane (Nguyen et al., 2021; Shirbhate et al., 2023). However, effective retention of probiotics on the oral epithelium can be challenged by necessary oral hygiene practices and even the natural salivary flow, which dynamically changes the conditions for colonization (Van Holm, Carvalho, et al., 2023). Unlike antimicrobial therapies, even prolonged probiotic use does not produce adverse side effects (Nguyen et al., 2021; Rebelo et al., 2023).

Although probiotics alone do not provide a curative treatment, they aid the immune system, support pathogen elimination and restore homeostasis, thus contributing to restoration of oral health (Rebelo et al., 2023).

The effects of probiotics are enhanced by prebiotics, commonly arabinose, xylose and xylitol (Bernaś and Kłosek, 2024; Shirbhate et al., 2023). This combination of live and non-living components is termed a synbiotic (Duane et al., 2023). Synbiotics increase the quantity and survival of commensal microorganisms adhering to the oral epithelium, exhibiting higher effectiveness than either of the components alone (Inchingolo et al., 2023; Shirbhate et al., 2023). Research shows that synbiotics improve the stability of probiotic bacteria as they pass through the upper gastrointestinal tract (Mohd Fuad et al., 2023).

Systemic diseases, such as inflammatory bowel disease, cardiovascular, and respiratory conditions often originate from an unstable oral microbiome (Rebelo et al., 2023; Teles et al., 2022). Studies have also linked periodontal tissue disease to Alzheimer's disease, certain types of cancer, multiple sclerosis, pregnancy complications, and diabetes (Hardan et al., 2022; Rebelo et al., 2023; Teles et al., 2022). In recent years, the use of probiotics has been widely studied, as they demonstrate the ability to control dysbiosis or imbalance between the microbiome and the host (Chen et al., 2023; Rebelo et al., 2023). Probiotic therapy functions as a complementary treatment by stimulating the host's inflammatory response, however, it does not serve as the primary defense against oral diseases (Rebelo et al., 2023).

In this article, we present research confirming the positive role of probiotics and synbiotics as adjunctive therapies in individuals with periodontitis.

Due to small fluctuations in the temperature (37 degrees), humidity and pH (6.5-7.5), as well as available content and ability to combat pathogenic exogenous organisms, the oral cavity is one of the four structures in the human body most frequently inhabited by microorganisms (bacteria, archaea, fungi, protists, parasites and viruses). Its disturbed homeostasis is strongly associated with the states of health and disease (Barrea et al., 2023; Di Stefano et al., 2023; Homayouni Rad et al., 2023; Lee et al., 2021; Nie et al., 2023; Rebelo et al., 2023). Oral microbiome includes approximately 700 species of prokaryotes, fungi, protozoa and viruses inhabiting the following: the gingival sulcus (especially anaerobic organisms), surfaces of the teeth, the buccal mucosa, the soft palate and vestibule, the tongue and tonsils (Di Stefano et al., 2023; Homayouni Rad et al., 2023; Lee et al., 2021; Nguyen et al., 2021; Nie et al., 2023). In healthy individuals, the main component of the oral microbiome forms a composition of biofilm (Deandra et al., 2023). Diversity of microbiological organisms can be determined according to the area of their habitation. Unlike the microbiome found in the buccal mucosa, that on the surface of teeth and tongue is characterized by a wide range of bacteria species (Rebelo et al., 2023). The ability to form colonies and persist on the structures of the oral cavity is enabled by a matrix, which consists of glucans and exopolysaccharides (EPS) and is created by the biofilm bacteria and surrounded by an extracellular polymer matrix (EPM) (Di Stefano et al., 2023; Rebelo et al., 2023).

A disease-free oral cavity achieves homeostasis between commensal bacteria, the host immune system and bacteria themselves (Rebelo et al., 2023). Commensal microorganisms in the body perform a protective function by modulating our immune system in the battle against pathogens, preventing their colonization on the surface of oral structures (e.g., *S. cristatus* secretes arginine deaminase protein), growth and development using secreted bactericidal substances including bacteriocins, which are also responsible for an increase in pH (for example *S. mutans*) and hydrogen peroxide (Di Stefano et al., 2023; Homayouni Rad et al., 2023; Inchingolo et al., 2023; Nguyen et al., 2021; Rebelo et al., 2023). Hydrogen peroxide can be secreted due to the presence of various enzymes, e.g., by lactate oxidase, pyruvate oxidase, NADH-flavin-dependent reductase or by NADH oxidase (Nguyen et al., 2021). Some of the microbes (*Actinomyces*) have the ability to convert nitrates into ammonia, thanks to which the pH increases and the number of anaerobic bacteria responsible for periodontal diseases decreases (Rebelo et al., 2023). According to the "ecological plaque theory", commensal microorganisms inhabiting a healthy oral cavity transform into pathogens as a result of antagonistic or synergistic actions between groups, including the dominant growth of specific species that disrupt the balance in the host organism (Inchingolo et al., 2023; Rebelo et al., 2023; Shirbhate et al., 2023). The dominant bacterial species of the microbiome are *Actinobacteria*, *Proteobacteria, Fusobacteria, Bacteroidetes, Spirochaetes, Firmicutes,* as well as*, Prevotella, Streptococcus, Leptotrichia, Veillonella, Rothia, Corynebacterium, Capnocytophaga*, *Selenomonas*, *Treponema*, *Haemophilus* (Di Stefano et al., 2023; Homayouni Rad et al., 2023). A decrease in microbial diversity, which can lead to a change in metabolic activity and stimulate the onset of disease, is called dysbiosis (Di Stefano et al., 2023).

Probiotics play a supporting role in anti-inflammatory processes. They colonize the oral epithelium in an amount that displaces pathogenic bacteria (Deandra et al., 2023). They restore homeostasis between the microbiome and the host as a result of inhibiting pathogenic microorganisms (Di Stefano et al., 2023; Van Holm, Carvalho, et al., 2023).

A characteristic feature of the microbiome in oral diseases is reduced bacterial diversity and the dominance of pathogenic microorganisms without sufficient host immune response (Haas et al., 2021; Homayouni Rad et al., 2023). To maintain a healthy oral microbiome, probiotic bacteria modify the bacterial flora by secreting bactericidal substances (antioxidants), hydrogen peroxide (including *S. gordonii*), reuterin (*L. reuteri*), nisin (*L. lactis*) and reutericycline, lactic acid, lowering the pH of saliva, which hinders the formation of dental plaque and calculus. Thus, they compete with pathogens for a place of adhesion to the oral mucosa, reducing IL-17, IL-1b and TNF-alpha in gingival fluid and regulating the inflammatory receptor TLR4 (Ausenda et al., 2023; Bodke and Jogdand, 2022; Deandra et al., 2023; Homayouni Rad et al., 2023; Karaca et al., 2022; Lee et al., 2021; Rebelo et al., 2023; Shirbhate et al., 2023).

## PROBIOTICS

The definition of probiotics was finally established in 2003 (Shirbhate et al., 2023). Recently, probiotics have been the subject of many scientific studies. These living organisms, taken in appropriate amounts, have a beneficial effect on the proper functioning of the intestines, respiratory system, cardiovascular system, female urinary system and the oral cavity (Chen et al., 2023; Hardan et al., 2022; Homayouni Rad et al., 2023; Inchingolo et al., 2023; Kaźmierczyk-Winciorek et al., 2021; Rebelo et al., 2023; Teles et al., 2022). They have been present in many food products for many years now and in order to benefit from their functions, they have to be properly prepared and selected precisely for a specific disorder (Bodke & Jogdand, 2022; Van Holm, Lauwens, et al., 2023). More and more studies show their excellent effects in the prevention, treatment and control of diseases by maintaining homeostasis and normalizing the microclimate of digestive system (Hijová, 2024; Homayouni Rad et al., 2023; Lee et al., 2021). Probiotics contain bacteria, not harmful to a patient with a properly functioning immune system, and their safety has been confirmed by in vitro and in vivo studies (Kaźmierczyk-Winciorek et al., 2021).

Their action can be attributed to the inhibition of NF-kB pathways and the activation of regulatory T cells (Chen et al., 2023). It has been proven that some of the bacteria that inhabit the oral cavity have probiotic properties, including the most well-known and most commonly used, such as Lactobacillus species, Bifidobacterium (LAB), as well as Saccharomyces, Enterococcus, Streptococcus, Pediococcus, Leuconostoc, Bacillus, Escherichia genera (Barrea et al., 2023; Bodke and Jogdand, 2022; Di Stefano et al., 2023; Hardan et al., 2022; Shirbhate et al., 2023). When selecting a probiotic preparation, it is important to find a species that adheres to the oral mucosa and not only to the distal parts of the digestive tract (Rebelo et al., 2023). Preparations rich in these strains are sometimes supplemented with amino acids, minerals and vitamins (Barrea et al., 2023). There are also species with a broad spectrum of action, such as Lactiplantibacillus pentosus KCA1, Lactiplantibacillus plantarum WCFS1, Lacticaseibacillus rhamnosus GG and Limosilactobacillus reuteri AMBF471, which can be successfully used in many periodontal tissue problems (Van Holm, Carvalho et al., 2023).

So far, not all the effects of probiotics on the oral microbiome have been confirmed. It is known that probiotics act both indirectly and directly (Homayouni Rad et al., 2023) and their key functions include:

- indirect action:
    - regulation of permeability and colonization of the oral epithelium by non-pathogenic microorganisms;
    - enhancement of the local and systemic immune and non-immunological response of the host;
- direct action:
    - control of the composition of microorganisms through inhibiting the proliferation of pathogens and their bactericidal effect;
    - inhibition of the production of proinflammatory cytokines (IL-12, TNF-alpha) by pathogens;
    - production of immunoglobulins (sIgA), antioxidants, organic acids, fatty acids, hydrogen peroxide;
    - inhibition of the synthesis of IL-1beta, matrix metalloproteinases-8 (MMP-8), tissue inhibitor of metalloproteinase-1 (TIMP-1);
    - competition for adhesion surfaces in the process of dental plaque formation and nutrients;
    - creation of biofilm, by controlling the binding of microorganisms to proteins (Homayouni Rad et al., 2023; Inchingolo et al., 2023; Kaźmierczyk-Winciorek et al., 2021; Lee et al., 2021; Nguyen et al., 2021; Rebelo et al., 2023; Shirbhate et al., 2023).

The action of probiotics is not only associated with direct competition against pathogens, but also or primarily with supporting immunological processes occurring in the attacked places (Inchingolo et al., 2023). In addition to the above actions, probiotics stimulate cellular and systemic immune response (Shirbhate et al., 2023). Some of them, called psychobiotics, are known to regulate the brain-intestine axis (Kaźmierczyk-Winciorek et al., 2021).

Patients with periodontal disease may take probiotics in various forms, such as tablets, mouthwashes and toothpastes (Shirbhate et al., 2023). Whereas, individuals with a weakened immune system, including the elderly, organ transplant recipients, infants and pregnant women, should use postbiotics which are a combination of non- viable bacteria, cellular structures of microorganisms and a metabolic product, applied to limit the possibility of delivery of pathogenic genes and genes responsible for resistance (Hijová, 2024; Homayouni Rad et al., 2023; Shirbhate et al., 2023).

### PREBIOTICS

Prebiotics are indigestible substances, usually carbohydrates, not containing microorganisms. They enhance the activity and growth of commensal bacterial strains and, when combined with them, create synbiotics (Bernaś and Kłosek, 2024; Inchingolo et al., 2023; Shirbhate et al., 2023). Prebiotics stimulate the bactericidal and fungicidal effects of probiotics, help to increase the metabolic activity of commensal bacteria, allowing the host microbiome to maintain homeostasis (Bernaś and Kłosek, 2024; Mohd Fuad et al., 2023; Nguyen et al., 2021; Shirbhate et al., 2023). They are resistant to digestion and are not absorbed through the intestinal mucosa (Kaźmierczyk-Winciorek et al., 2021; Nguyen et al., 2021; Rebelo et al., 2023). Prebiotic ingredients are often included in food products to enrich their nutritional value (e.g., lactulose, fructooligosaccharides, starch, galactooligosaccharides) (Kaźmierczyk-Winciorek et al., 2021; Mohd Fuad et al., 2023). One study demonstrated the beneficial effects of the prebiotic N-acetyl-d-mannosamine on the composition of the oral microbiome. It increased the number of *Streptococcus* species (*S. mitis, S. oralis, S. sanguinis* and *S. gordonii*) (Nguyen et al., 2021). They have a positive impact on health since, among other things, they affect the lipid profile by reducing low-density lipoprotein (LDL), stimulate the immune response, help the growth of lactic acid bacteria, modulate the proper functioning of probiotics and affect the condition of gastrointestinal duct (Kaźmierczyk-Winciorek et al., 2021).

### PYNBIOTICS

The combination of a prebiotic with a probiotic creates a relatively recently defined synbiotic (Duane et al., 2023). Both of its components act synergistically and complementarily (Bernaś and Kłosek, 2024; Shirbhate et al., 2023). They increase the amount and survival rate of commensal microorganisms that adhere to the oral epithelium, and they are less effective when acting separately than jointly (Inchingolo et al., 2023; Shirbhate et al., 2023). The form of a synbiotic brings an advantage visible in studies, consisting in increasing the durability of probiotic bacteria passing through the first section of the digestive tract. We know the most successful prebiotics (arabinose, xylose and xylitol) and probiotics (*L. fermentum, L. plantarum* and *L. paracasei*) which, in any combination, protect the oral microbiome against colonization by, among others, *P. gingivalis*, the most common periopathogen in periodontitis (Mohd Fuad et al., 2023). These preparations are also known for lowering blood pressure, improving the absorption of important macronutrients in our body, such as calcium, magnesium and phosphorus, and reducing cholesterol levels (Kaźmierczyk-Winciorek et al., 2021).

### PROBIOTICS IN GINGIVITIS

In total, 90% of the world's population suffer from gingivitis, which begins with the deposition of supragingival plaque in the gingival sulcus or in its vicinity (Deandra et al., 2023; Lundtorp Olsen et al., 2023). It is a reversible condition (Rathee and Jain, 2023).

The effect of a good therapy should be elimination of inflammation (Rathee and Jain, 2023). In the absence of proper oral hygiene and incorrect technique of mechanical plaque removal, there is a high probability of recurrence of periopathogen colonization (Modiri et al., 2023). In order to protect the population from this problem, steps have been taken to introduce probiotics into the therapy of gingivitis (Rathee and Jain, 2023). Studies have shown the positive effect of probiotics on the healing of gingivitis (Modiri et al., 2023).

A chewing gum enriched with two strains of *L. reuteri* provided relief in patients with moderate and severe gingivitis, however, the chewing gum was only administered as a supplement to scaling and root planing (Shirbhate et al., 2023). An interesting finding emerged from a 28-day study investigating the effects of probiotics on biofilm-induced gingivitis, during which the study group received *L. rhamnosus* PB01 DSM14870 and *L. curvatus* EB10 DSM32307 along with 491 mg of xylitol, and oral hygiene procedures were discontinued for half of the study period (14 days). In the placebo group, the levels of Lautropia, Prevotella, Fusobacterium and Selenomonas species were relatively higher after 28 days (when gingivitis had already been stabilized, after 14 days of proper oral hygiene) compared to the group receiving the probiotic. In the group taking the probiotic, the level of Rothia species was higher than in the control group. This study led to the conclusion that the microbial composition in stabilizing gingivitis had changed due to the *L. rhamnosus* and *L. curvatus* probiotics combined with xylitol, without any significant changes in plaque parameters or BOP (Rathee and Jain, 2023). The species *L. paracasei* also proved effective in curing gingivitis, successfully lowering the level of interleukin 1-beta and influencing the composition of dental plaque (Rebelo et al., 2023). A group of 28 adults, aged 20-35, who took fermented milk with the addition of the probiotic strain *L. casei shirota* for four weeks, had reduced values of markers of gingivitis, narrowed the gingival crevice, reduced inflammation of the tissue and reduced the volume of gingival crevice fluid and BOP (Hardan et al., 2022; Homayouni Rad et al., 2023). Similar changes were observed after adults used a chewing gum rich in *L. reuteri* strains (*ATCC PTA 5289* and *ATCC 55730*) once or twice a day (Homayouni Rad et al., 2023). A mouthwash containing 14 commensal bacteria of our microbiome, including *Bifidobacterium*, *Bacillus*, *Lactobacillus* and *Streptococcus*, showed satisfactory results after its use. It caused a reduction in the deposition of dental plaque and reduced bleeding of gingiva in diabetics (Duane et al., 2023). *B. animalis ZK-77* showed hydrogen peroxide production and *B. longum ZK-10* inhibited biofilm formation by 66%. *S. salivarius ZK-102* in combination with *B. animalis ZK-77* effectively prevented biofilm formation by the periopathogen *F. nucleatum* (Nie et al., 2023).

## PROBIOTICS IN PERIODONTITIS

Many studies have shown the beneficial effect of probiotics in the adjuvant therapy of periodontitis (Chen et al., 2023; Van Holm, Lauwens, et al., 2023). This infectious, multifactorial disease, causing destruction of tissues surrounding the tooth, is caused by a competition of commensal bacteria with periopathogens (Chen et al., 2023). The lack of control over the host's immune system and the secretion of excess cytokines that induce inflammation also play an important role in the process (Kaźmierczyk-Winciorek et al., 2021). The most common bacteria in this disease are red complex bacteria, including *Porohyromonas gingivalis, Tannerella forsythia, Treponema denticola,* and the non-red pathogen *Aggregatibacter actinomycetemcomitans* (Shirbhate et al., 2023). Pathogenic species lead to inflammation followed by gingival recession with an increase in the amount of gingival fluid, loss of periodontal attachment, formation of pockets and loss of the alveolar process bone (Hardan et al., 2022; Shirbhate et al., 2023). Proteins, which are excessively produced during inflammation, are an excellent breeding ground for pathogenic bacteria, which worsens the condition and deepens inflammation of the oral cavity (Hardan et al., 2022). Periodontitis is a disease manifested by bleeding on probing, formation of periodontal pockets and, in the advanced stage, also by a reduction in the strength of supporting tissues, tooth mobility and even tooth loss (Rebelo et al., 2023; Shirbhate et al., 2023). The gold standard of periodontal treatment is scaling and root planning (SRP) and if necessary surgical therapy, which can be supported by the use of probiotics (Chen et al., 2023; Hardan et al., 2022).

## GROWTH INHIBITION OF PERIODENTAL PATHOGENS

In an *in vitro* study, including patients suffering from periodontitis, *P. gingivalis, P. intermedia, Aggregatibacter actinomycetemcomitans* and *F. nucleatum* were subjected to bactericidal action of *Streptococci* and *Lactobacilli* species (Di Stefano et al., 2023). *S. salivarius K12* and *S. salivarius M18* exhibited anti-inflammatory effects, which could be seen in the reduced levels of IL-6 and IL-8 mediators (Deandra et al., 2023).

Effectiveness of *L. salivarius* action is directly responsible for the bactericidal activity in the main causes of periodontal diseases, including *P. gingivalis*, *P. intermedia* and *P. nigrescens*. According to the results of *in vitro* studies, it is achievable due to the lactic acid being secreted in the homolactic fermentation process (Ausenda et al., 2023; Lee et al., 2021). In fact, the specific strain *L. salivarius ZK-88*, has many positive effects on the oral epithelium. Characterized by, among others, high anti-inflammatory activity, high index of antibacterial activity and also effective inhibition of pathogenic microorganisms biofilm formation (Nie et al., 2023). It has been found that one of the pathogens of the red complex *T. forsythia*, present in subgingival plaque, is sensitive to the action of *L. salivarius WB21* in patients suffering from periodontitis (Hardan et al., 2022). A major threat to *P. gingivalis* itself is posed by *L. salivarius ZK-88* and *S. salivarius ZK-102* (Nie et al., 2023).

*L. plantarum CCFM8724* was more effective than treatment with chlorhexidine, as proven in the study by Zhang et al (Rebelo et al., 2023). Further, *L. plantarum WCFS1* in the agar study showed the most successful bactericidal effect on *P. gingivalis* and *F. nucleatum*, but weaker against *A. actinomycetemcomitans* (Van Holm, Carvalho, et al., 2023). *W. cibaria* was effective against the same pathogenic bacteria, but surprisingly, the well-studied strain *L. rhamnosus GG* did not show this effect (Rebelo et al., 2023). Intake of *W. cibaria CMU*, by 92 adults during an eight-week study, reduced the number of pathogenic bacteria *F. nucleatum* and *S. aureus* (Inchingolo et al., 2023). Strong activity against *P. gingivalis, F. nucleatum* and *A. actinomycetemcomitans* was demonstrated by *L. rhamnosus GG, L. pentosus KCA1, S. salivarius AMBR074, S. salivarius AMBR075, S. salivarius AMBR024* and *S. salivarius AMBR158* (Van Holm, Carvalho, et al., 2023). *L. reuteri* inhibited the growth and development of *A. actinomycetemcomitans, P. gingivalis* and *P. intermedia* (Homayouni Rad et al., 2023; Lee et al., 2021). The inhibition of growth of *A. actinomycetemcomitas* was found in the subgingival plaque tubes *in vitro* due to the strains *S. sanguinis, S. uberis* and *L. gasseri* (Homayouni Rad et al., 2023). In the study on the elimination of the pathogen *P. intermedia*, the following strains showed a satisfactory effect: *L. pentosus KCA1, S. salivarius AMBR074, S. salivarius AMBR075* and *S. salivarius AMBR024* (Van Holm, Carvalho, et al., 2023). *Lactobacillus* had an antibacterial effect through the synthesis of bacteriocins similar to those of *W. cibaria* and had a positive effect on the increase in E-cadherin expression on the gingival surface (Chen et al., 2023; Deandra et al., 2023).

*S. dentisani*, found in the oral cavity of patients not affected by periodontal disease, secretes a bacteriocin, which limits the development of *P. gingivalis* and *F. nucleatum* species (Deandra et al., 2023). Bactericidal hydrogen peroxide is secreted indirectly by proteins released by *L. delbrueckii STYM* (Nguyen et al., 2021).

*L. gasseri* and *L. fermentum* also are species found in patients without periodontitis (Homayouni Rad et al., 2023; Lee et al., 2021). The first one occurred to be effective against anaerobic pathogens, and *L. fermentum* copped with microaerophiles (Nguyen et al., 2021). Furthermore, probiotic strains of the *Bifidobacterium* species, including *B. lactis* and *B. infantis*, effectively combat pathogens associated with periodontal disease in supportive treatment (Deandra et al., 2023). For example, strains *B. lactis* and *L. brevis* inhibited the growth of anaerobic bacteria in studies on mice (Nguyen et al., 2021). *B. animalis subsp. lactis HN019* effectively stimulates the host's immune system. When administered in a study on rats with periodontal disease, it brought the bactericidal effect through the secretion of organic acids which evoked the destruction of Gram-negative bacterial membranes (Deandra et al., 2023; Nguyen et al., 2021).

## IMMUNE RESPONSE

*Lactobacillus*, which constitutes less than 1% of the oral microbiota, synthesizes the antioxidant substance KetoC, in HepG2 cells - acting on the nuclear erythroid 2- related factor 2-antioxidant response element (NRF2-ARE) pathway and in gingival tissue cells in the process of the G protein-coupled receptor on the 120-NRF ARE-MAPK pathway. It also secretes 10-hydroxy-cis-12-octadecenoic (HYA), which has anti-inflammatory effects by reducing inflammatory cytokines and decreasing the phosphorylation of ERK signal-dependent kinase (Deandra et al., 2023). Excellent results of the study on the host's immune response in periodontal treatment were presented by the combination of *B. longum BL986* strains with *L. rhamnosus LRH09*. Application of this combination of probiotics resulted in an increase in anti-inflammatory interleukin 10 and a decrease in pro-inflammatory cytokines and NF-kB (Chen et al., 2023).

The *L. helveticus SBT2171* strains are responsible for the weakened expression of TNF-alpha, interleukin 1beta and 6 factors in periodontitis caused by the pathobiont *A. actinomycetemcomitans* (Chen et al., 2023). *S. cerevisiae,* used alone or as a complementary element to SRP treatments, may be important for the level of alveolar bone loss, a decrease in TNF-alpha and IL-1beta factors, as well as an increase in the anti-inflammatory cytokine IL-10 in patients with periodontitis, which was studied on rats (Deandra et al., 2023; Nguyen et al., 2021). During the study on laboratory rats suffering from periodontitis, *B. animalis subsp. lactis HN019* showed the following results: it decreased the level of IL-1beta, impacted the expression of TNF-alfa and IL-6 (mediator of bone resorption in periodontitis) as *L. helveticus SBT2171*, decreased the levels of BD-3, TLR4, CD4 in the gingival tissue and the amount of RANKL-OPG (Deandra et al., 2023; Nguyen et al., 2021).

*Lactobacillus* species inhibit pro-inflammatory processes by increasing the presence of beta-defensin in the epithelium and decreasing the level of TIMP-1 (Chen et al., 2023). *Lactobacillus* bacteria affect the gene encoding IL-8 (CXCL8). As a result of its action, the amount of this interleukin increases, which has an impact on, among others, *P. gingivalis* bacteria (Nguyen et al., 2021). Inhibition of the secretion of Th17 lymphocytes is one of the actions of *Lactobacillus* in the treatment of chronic periodontitis (Kaźmierczyk-Winciorek et al., 2021). Resistance to *P. gingivalis* and *F. nucleatum* was acquired by organisms using the probiotic *L. acidophilus*, in the form of the production of immunoglobulin G antibodies in serum and A in saliva (Nguyen et al., 2021). Its strain LA5 is known to reduce virulence factors of such bacteria as *P. gingivalis* and *F.nucleatum* (Rebelo et al., 2023). The strain *B. animalis subsp. lactis HN019* effectively stimulates the host's immune system. Interestingly, in a study on rats, *B. subtilis*, through its action, consisting in mimicking pathogenic periodontal microorganisms, interacts with Toll-like receptors on dendritic cells, which affects the immune response of the diseased host (Nguyen et al., 2021). *B. lactis* and *L. kefiri* are species that affect the regulation of proinflammatory and anti-inflammatory cytokines (Chen et al., 2023). *S. dentisani* in patients without periodontal disease reduces the level of cytokines responsible for inflammation (Deandra et al., 2023).

## IMPACT ON ALVEOLAR BONE AND PERIODONTAL ATTACHMENT

In a study on rats with *B. animalis, subsp. lactis HN019*, the level of bone loss was reduced and additionally a decrease in the inflammatory mediators' levels was noted (Kaźmierczyk-Winciorek et al., 2021). A strain of the same species, *B. longum BL986,* taken together with *L. rhamnosus LRH09* reduced attachment loss and destruction of the alveolar bone in periodontal disease (Chen et al., 2023). *B. lactis* performed excellently in a study on rats concerning the immune control of bone tissue remodeling through action on nuclear factor kappa B. The reduced bone loss was also influenced by the strain *B. subtilis (CH201)* by lowering the activity of osteoclasts. This action was demonstrated by the C-terminal peptide, a marker of bone resorption, in a study on rats (Nguyen et al., 2021).

Various *Lactobacillus* strains prevent bone loss around the periodontal region (Nguyen et al., 2021). The *L. rhamnosus* species has a positive effect on the level of alveolar bone and is also known to alleviate apical periodontitis in animals (Chen et al., 2023; Nguyen et al., 2021). *L. brevis*,

owing to arginine deiminase, reduces bone resorption and also inhibits the production of nitric oxide, which reduces bone levels (Nguyen et al., 2021).

The combination of the two most popular probiotic species (*Lactobacillus* and *Bifidobacterium*) resulted in spectacular effects for the Plaque Index (PI), Modified Gingival Index (MGI), Bleeding Index (BI), Pocket Depth (PD) and Clinical attachment loss (CAL) indices - all indicated an improvement in the functioning of periodontal tissues (Hardan et al., 2022).

A 2017 study on a group of 22 patients provided satisfactory results. Following the use of probiotics rich in *L. brevis* and *L. plantarum*, periodontal pockets were visibly reduced in each applied area (Hardan et al., 2022). The influence of probiotic preparations containing *L. reuteri* brought a beneficial effect in as many as five important parameters, i.e., BOP, gingival index (GI), plaque index (PI), periodontal pocket depth and periodontal attachment level (Chen et al., 2023; Inchingolo et al., 2023). The same probiotic administered twice a day, in lozenges, reduced probing depth and provided a gain of connective tissue attachment (Ausenda et al., 2023). Strains *L. reuteri DSM17938* and *L. reuteri ATCCPTA5289* helped in shallowing inflammatory periodontal pockets (Lee et al., 2021). In a study with *L. reuteri*, the depth of periodontal pockets that required surgical treatment was assessed after 24 weeks. The difference in depth was 1 mm, which supports the thesis that this species may be effective in treating patients with deep pockets (Chen et al., 2023; Inchingolo et al., 2023). Patients, both those who received and did not receive SRP, after treatment with the probiotic *L. reuteri*, were also able to experience the effects of commensal bacteria through pocket reduction, as well as improved CAL, sulcus bleeding index (SBI) and BOP reduction. A similar effect was demonstrated by *L. sporogenes* treatment, however, in addition to reducing pocket depth, there was also a decrease in the amount of dental plaque and the periodontal attachment level was gained (Hardan et al., 2022). In a 90-day study, patients with severe periodontitis received a mixture of *L. reuteri, L. salivarius* and *L. acidophilus* strains. After that period, the depth of the pockets, CAL and BOP were examined and it was found that all the values had been reduced (Kaźmierczyk-Winciorek et al., 2021). All the four indices (CAL, PPD, BOP and PI) also improved after the use of the *L. rhamanosus SP1* strain (Hardan et al., 2022).

Unchanged microbiota composition, though with a visible improvement of periodontal condition, was observed among young adults after the intake of *B. animalis subsp. lactis BB-12* and *L. rhamnosus GG*(Homayouni Rad et al., 2023). The probiotic *B. animalis subsp. lactis HN019* caused a reduced depth of probing of pockets, dental plaque, immunocompetence of gingival tissues and increased levels of connective tissue attachment in patients with periodontitis.

## Summary

Periodontal disease, despite its high prevalence worldwide, continues to be a condition that, in many cases, leads to tooth loss due to inadequate oral hygiene.

Probiotics are becoming excellent substitutes for antibiotics in the treatment of periodontal disease, which helps to reduce the existing problem of bacterial resistance and stops the mechanism of commensal bacteria. There is no evidence from studies indicating a lack of benefit from supplementing the mechanical SRP method with probiotics, which makes it a valuable approach in providing protection against the most aggressive and severe stages of the disease.

Compared to antimicrobial therapy, the use of probiotics, even on a long-term basis, does not lead to side effects. It also strengthens the immunological response to pathogens without intensification of periodontal destruction. Apart from probiotics, also symbiotics, i.e., combinations of probiotics with non-digestible prebiotic elements, have become more and more popular.

Although probiotics do not fulfill a whole therapeutic function on their own, they certainly support the immune system by helping to eliminate pathogens and restore oral cavity homeostasis. Despite numerous studies conducted, it remains essential to assess the precise effects of long-term exposure to specific strains on the oral microbiome and thus identify the most beneficial approach for addressing periodontal disease.

## Bibliography


Ausenda F., Barbera E., Cotti E., Romeo E., Natto Z. S., Valente N.A. 2023. Clinical, microbiological and immunological short, medium and long-term effects of different strains of probiotics as an adjunct to non-surgical periodontal therapy in patients with periodontitis. Systematic review with meta-analysis. *The Japanese Dental Science Review* 59, pp. 62–103. DOI: 10.1016/J.JDSR.2023.02.001.

Barrea L., Verde L., Auriemma R.S., Vetrani C., Cataldi M., Frias-Toral E., Pugliese G., Camajani E., Savastano S., Colao A., Muscogiuri G. 2023. Probiotics and Prebiotics: Any Role in Menopause-Related Diseases? *Current Nutrition Reports 12*(1), pp. 83–97. DOI: 10.1007/S13668-023-00462-3.

Bernaś A.A., Kłosek S. 2024. The Use of Probiotic Preparations in Caries Prevention and Treatment. *Journal of Health Study and Medicine* 2024(1), pp. 39–58. DOI: 10.2478/JHSM-2024-0002.

Bodke H., Jogdand S. 2022. Role of Probiotics in Human Health. *Cureus* 14(11). DOI: 10.7759/CUREUS.31313.

Chen Y.W., Lee M.L., Chiang C.Y., Fu E. 2023. Effects of systemic Bifidobacterium longum and Lactobacillus rhamnosus probiotics on the ligature-induced periodontitis in rat. *Journal of Dental Sciences* 18(4), pp. 1477–1485. DOI: 10.1016/J.JDS.2023.04.013.

Deandra F.A., Ketherin K., Rachmasari R., Sulijaya B., Takahashi N. 2023. Probiotics and metabolites regulate the oral and gut microbiome composition as host modulation agents in periodontitis: A narrative review. *Heliyon* 9(2). DOI: 10.1016/J.HELIYON.2023.E13475.

Di Stefano M., Santonocito S., Polizzi A., Mauceri R., Troiano G., Lo Giudice A., Romano A., Mascitti, M., Isola G. 2023. A Reciprocal Link between Oral, Gut Microbiota during Periodontitis: The Potential Role of Probiotics in Reducing Dysbiosis-Induced Inflammation. *International Journal of Molecular Sciences* 24(2). DOI: 10.3390/IJMS24021084.

Duane B., Yap T., Neelakantan P., Anthonappa R., Bescos R., McGrath C., McCullough M., Brookes, Z. 2023. Mouthwashes: Alternatives and Future Directions. *International Dental Journal* 73 Suppl 2(Suppl 2), pp. S89–S97. DOI: 10.1016/J.IDENTJ.2023.08.011.

Haas A.N., Furlaneto F., Gaio E.J., Gomes S.C., Palioto D.B., Castilho R.M., Sanz M., Messora M.R. 2021. New tendencies in non-surgical periodontal therapy. *Brazilian Oral Research* 35(Supp 2), pp. 1–18. DOI: 10.1590/1807-3107BOR-2021.VOL35.0095.

Hardan L., Bourgi R., Cuevas-Suárez C.E., Flores-Rodríguez M., Omaña-Covarrubias A., Nicastro M., Lazarescu F., Zarow M., Monteiro P., Jakubowicz N., Proc P., Lukomska-Szymanska M. 2022. The Use of Probiotics as Adjuvant Therapy of Periodontal Treatment: A Systematic Review and Meta-Analysis of Clinical Trials. *Pharmaceutics* 14(5). DOI: 10.3390/PHARMACEUTICS14051017.

Hijová E. 2024. Postbiotics as Metabolites and Their Biotherapeutic Potential. *International Journal of Molecular Sciences* 25(10). DOI: 10.3390/IJMS25105441.

Homayouni Rad A., Pourjafar H., Mirzakhani E. 2023. A comprehensive review of the application of probiotics and postbiotics in oral health. *Frontiers in Cellular and Infection Microbiology* 13. DOI: 10.3389/FCIMB.2023.1120995.

Inchingolo F., Inchingolo A.M., Malcangi G., De Leonardis N., Sardano R., Pezzolla C., de Ruvo E., Di Venere D., Palermo A., Inchingolo A.D., Corriero A., Dipalma G. 2023. The Benefits of Probiotics on Oral Health: Systematic Review of the Literature. *Pharmaceuticals (Basel, Switzerland)* 16(9). DOI: 10.3390/PH16091313.

Karaca B., Yilmaz M., Gursoy U.K. 2022. Targeting Nrf2 with Probiotics and Postbiotics in the Treatment of Periodontitis. *Biomolecules* 12(5). DOI: 10.3390/BIOM12050729.

Kaźmierczyk-Winciorek M., Nędzi-Góra M., Słotwińska S.M. 2021. The immunomodulating role of probiotics in the prevention and treatment of oral diseases. *Central-European Journal of Immunology* 46(1), pp. 99–104. DOI: 10.5114/CEJI.2021.104412.

Lee Y., Yoon Y., Choi K.H. 2021. Probiotics-Mediated Bioconversion and Periodontitis. *Food Science of Animal Resources* 41(6), pp. 905–922. DOI: 10.5851/KOSFA.2021.E57.

Lundtorp Olsen C., Massarenti L., Vendius V.F.D., Gürsoy U.K., Van Splunter A., Bikker F.J., Gürsoy M., Damgaard C., Markvart M., Belstrøm D. 2023. Probiotics Support Resilience of the Oral Microbiota during Resolution after Experimental Gingivitis-A Randomized, Double-Blinded, Placebo-Controlled Trial. *Nutrients* 15(22). DOI: 10.3390/NU15224805.

Modiri S., Heidari M., Shahmohammadi R., Jabbareh L., Maboudi A., Moosazadeh M., Vali H., Noghabi K.A. 2023. A tangible prospect for the treatment of gingivitis using a potentially probiotic strain Lactobacillus plantarum MK06 isolated from traditional dairy products: a triple blind randomized clinical trial. *BMC Oral Health* 23(1). DOI: 10.1186/S12903-023-03494-X.

Mohd Fuad A.S., Amran N.A., Nasruddin N.S., Burhanudin N.A., Dashper S., Arzmi M.H. 2023. The Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics in Oral Cancer Management. *Probiotics and Antimicrobial Proteins* 15(5), pp. 1298–1311. DOI: 10.1007/S12602-022-09985-7.

Nazir M.A. 2017. Prevalence of periodontal disease, its association with systemic diseases and prevention. *International Journal of Health Sciences* 11(2), article number 72. Available online: https://pmc.ncbi.nlm.nih.gov/articles/PMC5426403/.

Nguyen T., Brody H., Radaic A., Kapila Y. 2021. Probiotics for periodontal health-Current molecular findings. *Periodontology 2000* 87(1), pp. 254–267. DOI: 10.1111/PRD.12382.

Nie Q., Wan X., Tao H., Yang Q., Zhao X., Liu H., Hu J., Luo Y., Shu T., Geng R., Gu Z., Fan F., Liu Z. 2023. Multi-function screening of probiotics to improve oral health and evaluating their efficacy in a rat periodontitis model. *Frontiers in Cellular and Infection Microbiology* 13. DOI: 10.3389/FCIMB.2023.1261189.

Rathee M., Jain P. 2023. Gingivitis. *Australian Journal of Pharmacy* 96(1141), pp. 64–67. DOI: 10.1902/jop.1955.26.3.173.

Rebelo M.B., Oliveira C.S., Tavaria F.K. 2023. Novel Strategies for Preventing Dysbiosis in the Oral Cavity. *Frontiers in Bioscience (Elite Edition)* 15(4). DOI: 10.31083/J.FBE1504023.

Shirbhate U., Bajaj P., Chandak M., Jaiswal P., Sarangi S., Suchak D., Bharti L. 2023. Clinical Implications of Probiotics in Oral and Periodontal Health: A Comprehensive Review. *Cureus* 15(12). DOI: 10.7759/CUREUS.51177.

Teles F., Collman R.G., Mominkhan D., Wang Y. 2022. Viruses, periodontitis, and comorbidities. *Periodontology 2000* 89(1), pp. 190–206. DOI: 10.1111/PRD.12435.

Van Holm W., Carvalho R., Delanghe L., Eilers T., Zayed N., Mermans F., Bernaerts K., Boon N., Claes I., Lebeer S., Teughels W. 2023. Antimicrobial potential of known and novel probiotics on in vitro periodontitis biofilms. *NPJ Biofilms and Microbiomes* 9(1). DOI: 10.1038/S41522-023-00370-Y.

Van Holm W., Lauwens K., De Wever P., Schuermans A., Zayed N., Pamuk F., Saghi M., Fardim P., Bernaerts K., Boon N., Teughels W. 2023. Probiotics for oral health: do they deliver what they promise? *Frontiers in Microbiology* 14. DOI: 10.3389/FMICB.2023.1219692.

UMEDICAL REPORTS

# DERMATOLOGIC MANIFESTATIONS ASSOCIATED WITH COLORECTAL CANCER: SCOPING REVIEW

## OBJAWY DERMATOLOGICZNE ZWIĄZANE Z RAKIEM JELITA GRUBEGO: PRZEGLĄD

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**Streszczenie**: Objawy dermatologiczne są rzadką, ale bardzo zauważalną manifestacją rozwoju raka, takie obserwacje mają ważną wartość diagnostyczną w wykrywaniu raka. W niniejszym artykule przeanalizowano szereg możliwych objawów skórnych silnie związanych z przerzutami raka jelita grubego. Przedstawiamy również dermatologiczne objawy zaburzeń genetycznych i niegenetycznych oraz nietypowych infekcji, które są związane z rakiem jelita grubego. Obraz kliniczny przerzutów jest bardzo zróżnicowany i stanowi wyzwanie diagnostyczne dla klinicystów. Niniejszy artykuł zawiera kompleksowy wybór najczęstszych powiązanych obserwacji dermatologicznych w celu ułatwienia wczesnego wykrywania i szybkiej reakcji na raka jelita grubego.

**Słowa kluczowe*:*** rak jelita grubego, zmiany skórne, przerzuty, objawy dermatologiczne, zespoły paranowotworowe

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**Acronyms**:

AN – scanthosis nigricans
BRRS – Bannayan-Riley-Ruvalcaba syndrome
CRC – colorectal cancer
CCS – Cronkhite-Canada syndrome
CS – Cowden syndrome
DM – dermatomyositis
EGFR – epidermal growth factor receptor
GI – gastrointestinal
HNPCC – hereditary non-polyposis colorectal cancer syndrome
LS – Lynch syndrome
LTS – Leser-Trélat syndrome
MTS – Muir-Torre syndrome
NCCN – National Comprehensive Cancer Network
PJS – Peutz-Jeghers syndrome

## 1. Introduction

Dermatological manifestations are definitely not the first symptoms associated with colorectal cancer. Nevertheless, although they may seem unrelated to the disease, they may actually be a red flag being waved to alert us to pathological changes developing in the colon. Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and is correlated with a high mortality rate (Ferlay et al., 2015, Chirikian et al., 2021). Among patients affected by the disease, 20% have metastases and over 40% relapses (Biller and Schrag, 2021).

As the population age increases and screening methods improve, more patients are diagnosed with colorectal cancer (Pasquereau-Kotula et al., 2018, Patel et al., 2022). Colorectal cancer rarely metastasizes into the cutaneous tissue. Skin manifestations may occur both in the course of cancer development and years after surgical resection, and, if present, they usually indicate a poor prognosis as almost two-thirds of patients who develop them have a six-month survival rate (Bittencourt et al., 2018). Therefore, it is necessary to understand the nature of these lesions better in order to initiate prompt cancer management.

Age, genetic factors, diet, lifestyle, and environmental factors all have an impact on the occurrence of colorectal cancer. It is reported that over 25% of cancer cases is related to genetic familial history, and many of genetic diseases manifest themselves through characteristic skin lesions (Cooper et al., 2010). This review describes the most common macroscopic and microscopic cutaneous manifestations observed in metastatic colorectal cancer. It presents hamartomatous polyposis syndromes, such as Peutz-Jeghers syndrome (PJS), Cowden syndrome (CS), and Bannayan-Riley-Ruvalcaba syndrome (BRRS), as well as hereditary non-polyposis colorectal cancers, including Lynch syndrome (LS) and Muir-Torre syndrome (MTS). Additionally, it covers other paraneoplastic syndromes, i.e., dermatomyositis, Leser-Trélat syndrome (LTS), Cronkhite-Canada syndrome (CCS), and gangrene infection caused by Clostridium septicum, uniquely associated with colorectal cancer due to the tumor microenvironment.

Furthermore, enhanced cancer screening protocols are considered and improved surgical techniques preventing tumor adhesion on the skin are discussed.

## 2. Materials and methods

A systematic electronic search was performed on the PubMed database. The analysis included all data up to March 8, 2023. The search strategy was based on a combination of the keywords: “colorectal cancer and skin lesions” OR “dermatologic manifestations of colorectal metastasis” OR “colon and skin” AND “paraneoplastic dermatologic manifestation”. Hand-search was also carried out for additional studies on relevant topics.

The scoping review includes studies reporting the association between colorectal cancer and dermatologic symptoms, case studies and systematic reviews. It excludes references from conference papers, animal studies, papers in languages other than English or articles published before 1980.

PRISMA guidelines were followed and displayed in the flow chart in Fig. 1. The first and second authors collaborated independently in the screening process of the articles. A total of 530 records were identified on the database by using the keywords listed above. A majority of the duplicates identified were case study papers and they were removed accordingly.

One hundred and seventeen articles were read by the authors. Some of the records were rejected if any of the following exclusion criteria was met: repetitive case observations, irrelevant study type, irrelevant outcomes (studies focused purely on dermatology), or insufficient data (studies around genetic colorectal diseases with insufficient cases correlating CRC to dermatologic lesions). Eventually, 75 articles fulfilling the inclusion criteria were selected.

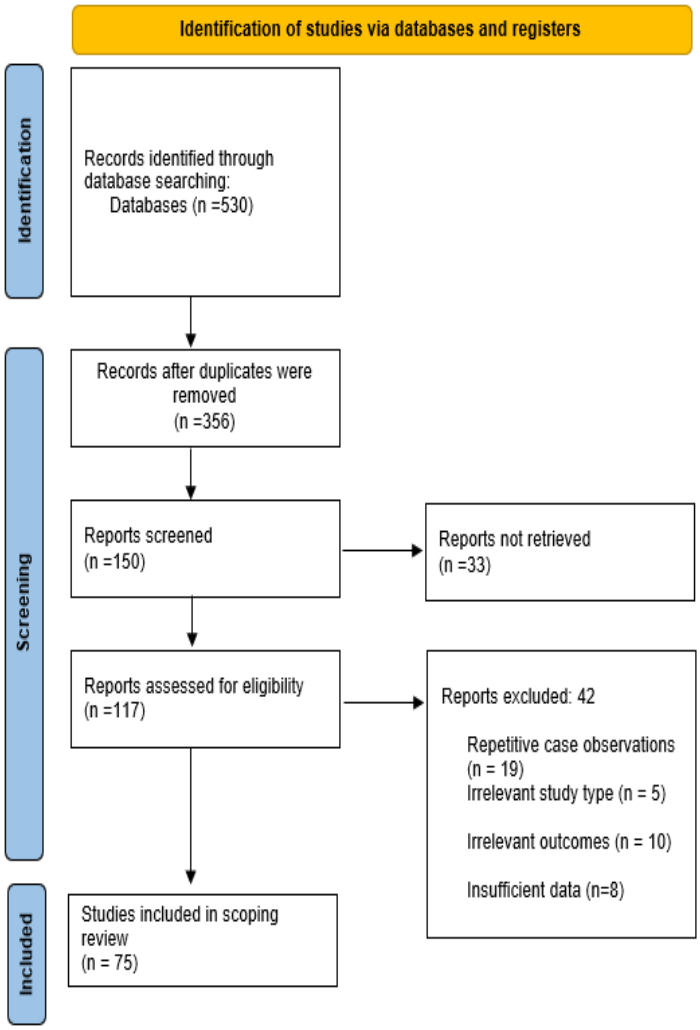


**Figure 1.** PRISMA Flow Diagram.

## 3. Results

### *3.1. Metastasis of colorectal cancer to the skin*

#### *3.1.1. Macroscopic and microscopic dermatological observations of metastatic colorectal cancer*

Cases of cutaneous metastases of colorectal cancer, resulting from an advanced stage and spread of the disease, are rare. It is reported that they occur in about 4 to 6% of patients (Bittencourt et al., 2018). The process of skin lesions can be caused by postoperative scars or it can be the first sign of colorectal cancer metastasis. In most cases, skin manifestations were perceived long before colorectal malignancy developed and could be the first indication of silent malignancy occurrence. The average time between the recognition of the skin manifestation and the death of a patient was around 14.8 months (Hakami et al., 2020).

However, there is also evidence which confirms that after skin metastasis is diagnosed, prognosis is poor and the average time of survival ranges from 18 to 34 months (Yang et al., 2020). That is probably due to cancer already spreading beyond the skin and invading many other organs.

It is also known that skin manifestations can develop about two to four years after a surgery, such as hemicolectomy or resection of the primary tumor (Hakami et al., 2020, Rajan et al., 2012).

Skin manifestations generally occur at several sites, the most common of which are the abdominal skin or wall, head, pelvis, back, neck, chest, scapula, parts of the upper extremities (e.g., hands) and lower extremities (knees, thighs and legs (Yang et al., 2020). There also reports on lesions present in other locations of the skin, i.e., along the lines of the scalp, on the face, ankles and genitalia, e.g., the scrotum or the groin (Nesseris et al., 2013; Yang et al., 2020).

Tumors that develop on the skin have a distinctive appearance, primarily because of the cancer's lack of differentiation and its ability to undergo transdifferentiation. (Hakami et al., 2020). The most common cutaneous manifestations are nodules, both painless and painful. The lesions may also appear intramuscularly or intraperitoneally, depending on the location of metastasis (Malla et al., 2019). The second most common presentation is the appearance of a cutaneous tumor, most often on the abdominal wall. One of the signs observed on dermatological examination are ulcerated tumors with granular and bleeding bases (Bittencourt et al., 2018). There are also some more advanced presenting as fast-growing painless flesh-colored nodules and hyperkeratosis (Hakami et al., 2020).

The list of dermatological observations also includes subcutaneous nodules, annular erythema, non-healing ulcers, blisters, alopecia patches, lesions resembling herpes-zoster or cellulitis, epidermal cysts, condylomas, and elephantiasis nostras verrucose (Fig. 2) (AlSubait et al., 2021; Bittencourt et al., 2018).

More advanced cutaneous manifestations may also be observed, such as erythematous multinodular cauliflower-like lesions (Nesseris et al., 2013). There is also some evidence of plaques, red or follicular papules, draining sinuses, simple cutaneous rash and cutaneous necrosis (AlSubait et al., 2021).

Microscopic examinations reveal typical neoplastic features, such as pleomorphism, hyperchromatic nuclei, prominent nucleoli, and various necrotic stages. Histopathologically, cutaneous metastasis of colorectal origin characteristically exhibits the presence of glandular structures with intervening desmoplastic stroma (Kemal et al., 2018).

If microscopic evaluation is limited due to poor differentiation of the neoplastic cells, immunohistochemical tests for CK7, CK20, CK19, and CDX2 can aid the diagnostic process, particularly when cutaneous manifestations are the primary presentation in the patient (Sadler et al., 2020).

Clinically, patients complain of pain, edema, and itching that these cutaneous lesions cause (Yang et al., 2020). Undoubtedly, these skin manifestations occur commonly in many other diseases. Therefore, each lesion should be examined very carefully by a dermatologist before it is diagnosed that it is the first sign of colorectal cancer (Yang et al., 2020).

**Table 1**. Macroscopic cutaneous manifestations.

| Most Common | Second Common | Others | More advanced |
|---|---|---|---|
| Nodules | Cutaneous tumors | Hyperkeratosis<br>Annular erythema<br>Non-healing ulcers<br>Blisters<br>Alopecia patches<br>Lesions resembling herpes-zoster or cellulitis<br>Epidermal cysts<br>Condylomas<br>Elephantiasis nostras verrucose | Erythematous multinodular lesions<br>Red or follicular papules<br>Draining sinuses<br>Cutaneous necrosis |

*3.1.2. Routes of colorectal cancer metastasis into the cutaneous tissue*

Cutaneous metastasis from colorectal cancer is extremely rare, and it usually appears years after the diagnosis or resection of the primary tumor. The most common sites to which CRC metastasizes are the lungs and the liver, while cutaneous metastatic lesions occur in 4-6.5% of cases, with the abdominal skin being the most common site. Preferentially, metastases affect areas close to the primary tumor (Fig. 3) (Bittencourt et al., 2018).

In almost all cases, cutaneous metastasis is synonymous with an extensive spread of the disease and indicates poor prognosis. Metastases may develop in distant sites, on the overlying skin, in distant regions of the skin, or on surgical scars.

The exact mechanism of cutaneous metastasis from internal malignancies is unknown, however,
a few hypotheses have been proposed regarding this issue. Some of them include direct extension of neoplasm through the typical hematogenous or lymphatic spread. Colorectal cancer can also form metastases along the ligaments of embryonic origin, the urachus.

It is highly possible that colorectal tumor cells implant on the skin during an excision surgery (Kemal et al., 2018). The abdominal epithelium on surgical scars is often vulnerable to metastasis, and the most plausible explanation for that would be direct tumor implantation. This may occur during various procedures, such as hemicolectomy, or even biopsy. However, the issue has not yet been studied thoroughly and requires further consideration.

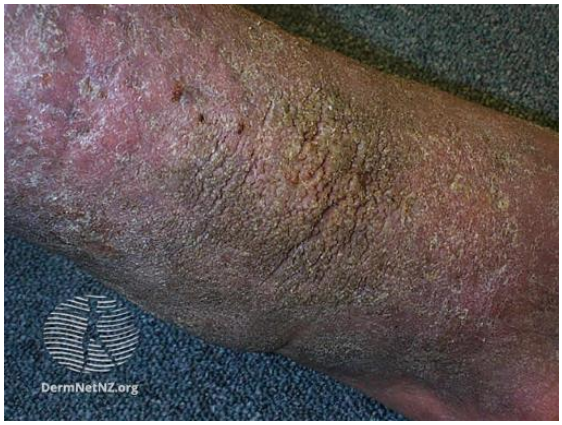


**Figure 2.** Elephantias nostras verucosa (DermNet, 2023).

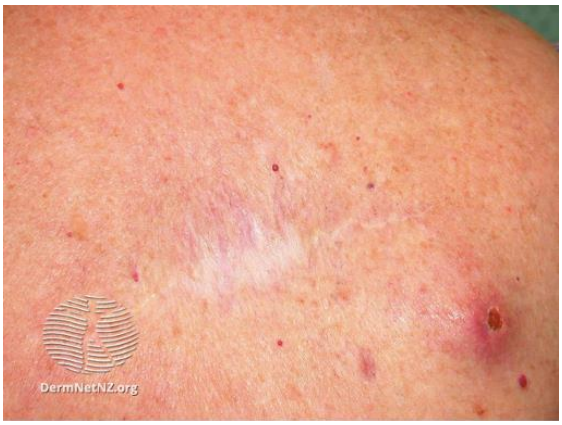


**Figure 3.** Cutaneous metastasis occurring near the excision site (DermNet, 2005).

### *3.1.3. Management of scars associated with tumor spread*

Skin manifestation of the cancer can be the first observable sign of metastasis (Hakami et al., 2020). To avoid spreading postoperative scars associated with colorectal cancer, it is necessary to ensure that they are managed properly. Medical specialists should work on developing methods that minimize invasive procedures performed during hemicolectomy and resection operations (Hakami et al., 2020; Rajan et al., 2012) since the main goal is to reduce the negative impact of surgery on a patient's body.

A procedure in oncological colon treatment that offers many advantages is laparoscopic surgery (Durak et al., 2022). Studies must be carried out to compare tumor implantation cases occurring following classical surgical resection vs. laparoscopic procedure. With current medical advancements, there several surgical techniques that may used, however, medical specialists should decide on the method applied individually, taking into consideration specific circumstances and preferences of each patient.

Advanced imaging techniques are needed to enhance the accuracy of surgical procedures and avoid tumor implantations (Kijima et al., 2014). Overall, future options of managing scars associated with colorectal cancer spread may involve a combination of improved surgical methods and advanced imaging techniques.

### *3.1.4. Colorectal cancer treatment and its dermatologic side effects*

Diagnosis of widespread cutaneous metastasis is associated with poor prognosis and short survival rates. If metastases are identified early, the treatment can be modified accordingly to improve prognosis (Bittencourt et al., 2018). Cancerous colorectal lesions can be resectable or unresectable. Those that are unresectable require intensive systematic treatment like chemotherapy or immunotherapy. Resection surgeries are followed by chemotherapy and radiotherapy (Biller and Schrag, 2021).

Patients presenting with KRAS/NRAS/BRAF wild-type metastatic colon cancer are more likely to live longer due to their sensitivity to chemotherapy combined with EGFR inhibitors, like cetuximab and panitumumab (Biller and Schrag, 2021). Typically, they can extend the survival time of the patient by approximately two months. Acneiform rash, also known as papulopustular rash, is one of the most common side effects of treatment with these EGFR inhibitors. It usually develops around the scalp, face, and trunk after two to four weeks of treatment initiation and disappears after eight weeks (Antonetti et al., 2022). Perforative collagenosis and erosive pustular dermatosis appear on the scalp and have been reported in patients taking panitumumab (Okuno et al., 2022, Tsutsui et al., 2021).

Patients returning to the clinic with cutaneous manifestations after surgical resection in multiple locations have very poor prognosis with a life expectancy of only six months. The previously mentioned therapies are not always effective and may have some negative effects. Patients are then referred to palliative care in addition to palliative radiation therapy (Nesseris et al., 2013).

## *3.2. Colorectal diseases directly associated with cancer and skin lesions*

### *3.2.1 Hamartomatous polyposis syndromes*

Hamartomatous polyposis syndromes are a group of diseases that present with numerous gastrointestinal (GI) polyps, dermatological lesions, and predisposition to GI cancers (Tab. 2). These syndromes are categorized based on histological presentation of polyps (Campos et al., 2015). This paper examines each syndrome separately, including their specific skin features, typical patient population, malignancy risk, and genetic background.

This paper does not cover the syndromes that lack characteristic skin lesions (juvenile polyposis syndrome, multiple endocrine neoplasia type 2B, Birt-Hogg-Dubbe syndrome, neurofibromatosis I, hereditary mixed polyposis syndrome).

### PEUTZ JEGHERS SYNDROME

Peutz Jeghers syndrome (PJS) is an autosomal dominant disease that presents with numerous hamartomatous GI polyps and mucocutaneous hyperpigmentation. The syndrome is caused by mutations in STK11 encoding a tumor suppressor, serine-threonine kinase and occurs equally in both genders and all racial groups. (Hemminki et al., 1998, Shakil et al., 2022)

Pediatric patients are usually identified during a routine check-up. They present characteristic dark blue/brown macules around the mouth, buccal mucosa, nostrils, and the eyes Fig. 2. These pigmentations usually disappear around adulthood. Thus, it is essential that detailed history of the patient is taken, including information about any abnormal skin pigmentations during childhood even if those lesions have disappeared a while ago (Chen et al., 2017, Miyahara et al., 2020).

PJS polyps can spread anywhere in the gastrointestinal system, increasing the risk of colorectal cancer up to 39% in patients with a mean age of 42-46 years old. The risk of intussusception among younger patients aged below 10 is 44%.(Van Lier et al., 2011)

According to the National Comprehensive Cancer Network NCCN 2021 guidelines, it is recommended that patients undergo molecular genetic studies to identify the STK11 mutation. Colonoscopy and endoscopic studies should start at the baseline age of 8 years old. If some polyps are detected then the patient must undergo repeated testing once in one to three years depending on the number, size, and histology of the resected polyps. Adult females are also instructed to undergo mammography beginning at the age of 30 years and routine gynecologic examination beginning at the age of 18 years. Males are advised to have a testicular ultrasound done starting from the age of 10 years if symptoms and polyps are found (Weiss et al., 2021).

## PTEN HAMARTOMA TUMOR SYNDROME: COWDEN SYNDROME (CS) AND BANNAYAN-RILEY-RUVALACABA SYNDROME (BRRS)

BRRS and CS are autosomal dominant diseases that occur due to a mutation in the tumor suppressor gene PTEN, highly predisposing to cancer development. Colonic polyps are found in up to 95% of patients with CS and BRRS (Heald et al., 2010). Both diseases present with characteristic dermatological manifestations that help doctors to identify them and prevent multisystem tumors affecting such organs as the colon, breast, thyroid, kidneys, and skin melanomas (Tan et al., 2012).

### COWDEN SYNDROME

Stanich et. al and Heald et. al reported presence of colonic polyps in over 90% of their patients with Cowden syndrome (Heald et al., 2010, Stanich et al., 2011). Most of the polyps were hamartomatous, some other types observed were hyperplastic, inflammatory, leiomyomas, adenomas, etc. (Smerdel et al., 2020).

The average age of diagnosing metastatic colorectal cancer in Cowden Syndrome patients is 47 years, and the lifetime risk is estimated to be two to three times higher than in the general population (Miyahara et al., 2020. Stanich et al., 2011).

Mucocutaneous manifestations develop by late 20's in 90% of individuals with CS. Many various types of lesions appear and indicate the risk of CS. The most characteristic ones are trichilemmomas, which are benign hamartomatous lesions of the hair follicle (Fig. 5). Other, also quite specific for the condition, are acral keratoses, oral papillomas, and mucocutaneous neuromas (Lim and Ngeow, 2021).

Close surveillance planned specifically for patients with Cowden syndrome is essential due to their increased risk of formation of benign and metastatic tumors. NCCN recommends that colonoscopy be regularly performed in CS patients every five years, beginning from the age of 35. Colonoscopy is necessary immediately if the patient develops symptoms as bleeding on defecation, diarrhea, and pain. If the patient is closely related to a relative previously diagnosed with colorectal cancer, then they should be closely monitored already at the age five to ten years younger than that at which their family member was diagnosed with the disease (Weiss et al., 2021). Following the proposed guidelines ensures optimal survival and treatment for the patients.

BANNAYAN-RILEY-RUVALCABA SYNDROME (BRRS)

BRRS is a rare pediatric autosomal dominant disease that presents quite similarly to CS and is also mostly linked to PTEN mutation. Most clinical data come from patients with CS and less commonly those with BRRS (Pilarski et al., 2013).

Hamartomatous polyps are observed in 35% to 45% of patients with BRRS. The lesions are located all over the gastrointestinal tract, particularly in the colon and rectum.

BRRS is usually diagnosed in infancy, unlike CS which prevails in adulthood. It presents primarily with pigmented macules on the toes and glands, subcutaneous lipomas, severe anemia, and macrocephaly (Boland et al., 2022). Pediatric patients may present with rectal bleeding, watery diarrhea, and abdominal pain. These symptoms are likely to arise when the polyp becomes larger (Erkek et al., 2005). Interestingly, intestinal hamartomas appear more symptomatic in patients with BRRS compared to CS.

Older studies have not associated BRRS polyps with an increased risk of malignancy (Erkek et al., 2005). However, recently Hendriks et al. have suggested annual testing of hemoglobin level beginning from infancy for early detection of hamartomas and have recommended the same surveillance protocol as that applied in the case of CS patients.

**Table 2**. Dermatologic features and colon cancer risk in hereditary non-polyposis syndrome and hamartomatous polyposis syndromes (based on the literature).

| Syndrome | Associated Gene | Main Dermatologic features | Mean age of colon cancer development | Colon cancer risk |
|---|---|---|---|---|
| Hereditary non--polyposis syndrome (HNPCC) (LS and MTS) | MLH1, MSH2, MSH6, PMS2 | Sebaceous lesions in the periocular region, sebaceous adenomas, keratoacanthomas | 20-40 | 80% |
| Hamartomatous polyposis syndromes: | | | | |
| Peutz-Jeghers syndrome (PJS) | STK11 | Characteristic dark brown macules around the mouth | 42-46 | 39% |
| Cowden and Bannayan Riley disease | PTEN | trichilemmomas, acral keratoses, oral papillomas | 47 | 9-16% |

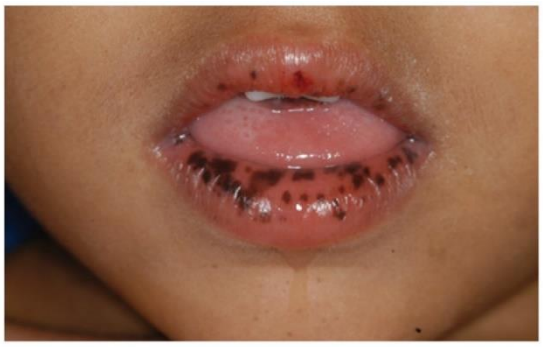

**Figure 4.** Peutz-Jeghers syndrome: black spots in the perioral area (image courtesy of Pablo Agustin Vargas).


**Figure 5.** Trichilemmomas on facial hair follicles in Cowden syndrome (DermNet, 2022a).

*3.2.2 Hamartomatous polyposis syndromes*

HEREDITARY NON-POLYPOSIS SYNDROME (HNPCC): LYNCH SYNDROME (LS) & MUIR-TORRE SYNDROME (MTS)

Lynch Syndrome (LS), formerly known as hereditary non-polyposis colorectal cancer syndrome (HNPCC), is a common hereditary cancer predisposition syndrome with a prevalence of 1 in 300. Without intervention, patients with HNPCC have an 80% lifetime risk of colorectal cancer with the onset at age of 20-40 years. (Aarnio et al., 1999)

Muir-Torre syndrome (MTS) is a subtype of HNPCC. Cancers of the GI tract make up more than 60% of the visceral malignancies in MTS, and colorectal cancer is the predominant neoplasm (Cohen et al., 1995, Cohen et al., 1991). Additionally, extraintestinal manifestations, such as sebaceous gland carcinomas, breast carcinomas, epitheliomas, and multiple or early-onset keratoacanthomas are also associated with MTS (Cohen et al., 1991, Pettey and Walsh, 2005, Strate and Syngal, 2005).

MTS is inherited as an autosomal dominant disease. It is caused by a germline mutation in a DNA mismatch repair gene. The most commonly mutated mismatch repair genes are MLH1, MSH2, MSH6, and PMS2 (Kudibal and Venzo, 2018). MTS more commonly involves a mutation of MSH2, while HNPCC shows an equal prevalence of MLH1 and MSH2 (Lazar et al., 2007). Thus, sebaceous tumor microsatellite instability and immunohistochemical staining for MSH2 help screen patients who are suspected of MTS.

The diagnostic criteria, proposed by Schwartz et al. in 1995, included the presence of a sebaceous tumor (sebaceous adenoma, sebaceous carcinoma, or sebaceoma) and at least one visceral malignancy (Schwartz and Torre, 1995). The current diagnosis of MTS is mostly clinical, history-based, and supplemented by immunohistochemistry and molecular analysis (Mathiak et al., 2002).

As regards the morphology of MTS, there is a large spectrum of sebaceous, from those benign, through hyperplastic to aggressive carcinomas. Sebaceous adenomas appear as slow-growing tan pink or yellow nodules and papules. They are often centrally umbilicate with ulceration (Fig. 6). Sebaceomas (sebaceous epithelioma) are similar to sebaceous adenomas but larger in size
(5-30mm) and have a higher content of basaloid cells (>50%) on immunohistochemistry studies. Sebaceous carcinomas more often appear on the periocular region and present as painless nodules without ulceration on the upper eyelid.

Burris et al. recommended that all patients with sebaceous neoplasms be screened for MTS. Family history was emphasized as the most cost-effective screening tool with the highest positive and negative predictive values. It should be taken first, before implementing other costly confirmatory tests (Burris et al., 2019).

Once the diagnosis of MTS is confirmed with genetic testing, annual evaluations for malignancy should be performed (Simic et al., 2021). Treatment applied may involve excision and adjuvant radiation therapy (Ponti and Ponz de Leon, 2005).

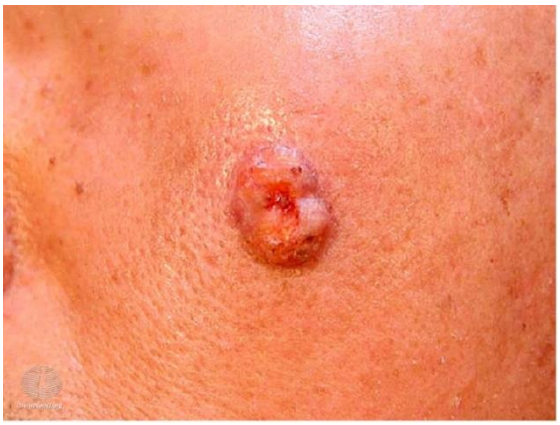

**Figure 6.** Sebaceous adenoma in Muir-Torre syndrome (DermNet, 2021a).

### *3.2.3 Other paraneoplastic syndromes*

#### LESER-TRÉLAT SYNDROME (LTS)

The Leser-Trélat syndrome (LTS) is a sudden occurrence of multiple seborrheic keratoses in association with an internal malignancy (Rampen and Schwengle, 1989). Most patients with “Sign of Leser-Trélat” develop associated adenocarcinomas. The most common locations of these cancerous lesions are the stomach, colon, and the rectum (Cohn and Classen, 1993, Ginarte et al., 2001, Heng et al., 1988). “Sign of Leser-Trélat” is not identical to LTS. It can be present with or without occult malignancy (Heaphy et al., 2000, Ponti et al., 2010), whereas LTS requires malignancy confirmation.

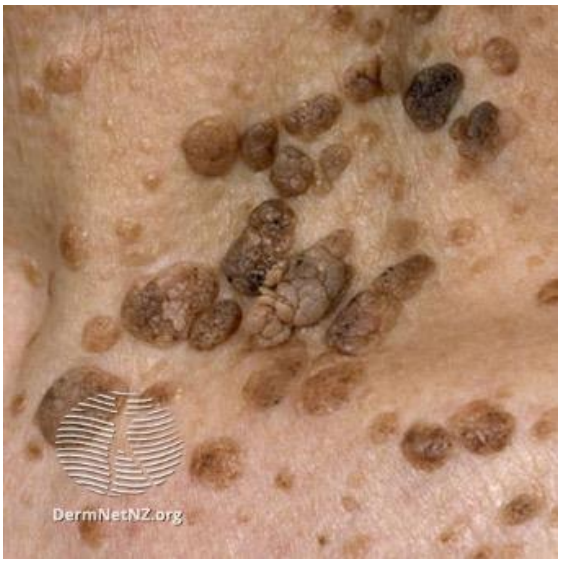


**Figure 7.** Multiple eruptive seborrheic keratoses (”sign of Leser-Trélat”) (DermNet, 2004).

Seborrheic keratoses are pigmented well-circumscribed lesions. They may be black or have natural or tan skin color (Fig. 7). Their texture is typically described as waxy, and velvety, and they may have a "stuck-on" appearance. Pruritus is a leading symptom associated with these lesions (Schwartz et al., 1991). They are mostly found on the trunk and the extremities and often resemble the “Christmas tree” or “raindrop” pattern on the patient’s back. The exact pathophysiology underlying the sign of Leser-Trélat remains unknown. Still, there is a strong suspicion that the release of growth factors and cytokines from the neoplasms stimulates the eruptive development of seborrheic keratoses. Particularly, overexpression of EGF-alpha and EGFR (epidermal growth factor receptor) may contribute to the eruptive characteristics of malignancies (Ellis et al., 1987). Theodropolous et al. reported that 44% of colorectal cancer patients (median age 64 years) show a high EGFR expression (Theodoropoulos et al., 2009). Additionally, Kim et al. demonstrated that a higher expression of EGFR is concurrent to a more advanced stage of CRC (Oh et al., 2011).

Lesions occurring in the Leser-Trélat syndrome are harmless and do not require specific treatment. They tend to resolve once the underlying disease is treated, therefore the primary aim of the physician is to detect and treat cancer. If necessary, such lesions can be removed using various surgical procedures.

It is noteworthy that about two-thirds of patients with LTS develop accompanying paraneoplastic symptoms, such as Acanthosis nigricans (AN). Malignant acanthosis nigricans most often occurs in the course of gastrointestinal adenocarcinomas (80%) (Gunduz et al., 2013, Lee et al., 2011). AN is characterized by symmetric, hyperpigmented, and velvety plaques (Phiske, 2014). A malignant type of AN is a more severe form of AN. It is associated with poorer prognosis and accompanied by paraneoplastic tripe palms (acanthosis palmaris, Fig. 8), strongly related to internal malignancy at 90%. Another well-known risk factor for colorectal cancer, particularly in males, is obesity (Kant and Hull, 2011). It is suspected that there may be comorbidity between obesity-related AN and colorectal cancer (Lee et al., 2011). Thus, the presence of pruritus, obesity, and acanthosis nigricans hyperpigmentation should majorly alert the physician of underlying malignancies.

LTS is usually ignored by both patients and practitioners, which results in a delayed diagnosis and unresectable lesions. Patients with LTS have a poor prognosis and an average survival rate of 10.6 months (Holdiness, 1987). The recommended diagnostic workup should include laboratory and histopathological tests, along with imaging examinations such as ultrasound, CT scans, or PET scans to detect any underlying tumors that may not be evident on a physical exam. The laboratory tests include routine complete blood count, chemistry tests and urinalysis. A Papanicolau test and

cervical cytological examination should be performed in women, and a prostate specific antigen (PSA) test in men. Awareness of the syndrome, as well as thorough workup may lead to earlier identification of the underlying malignancy, and thus a more favorable outcome (Constantinou et al., 2010).

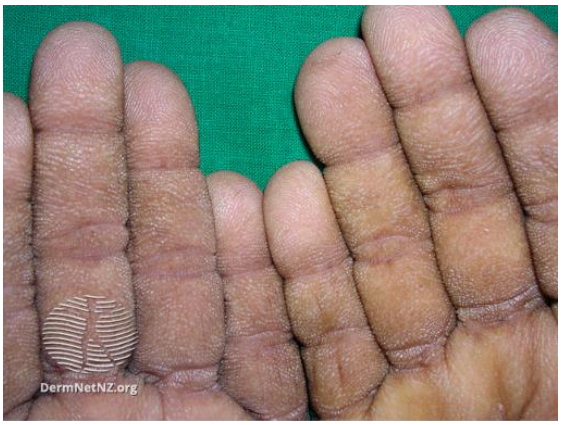


**Figure 8.** Tripe palms in paraneoplastic AN (DermNet, 2022b).

### DERMATOMYOSITIS

Dermatomyositis (DM) is an idiopathic inflammatory myositis manifested by skin rash, muscle weakness and aches (Gkegkes et al., 2018). Approximately 25% of DM patients present with a concurrent malignancy (Shah et al., 2013), affecting mostly the ovaries, colon and rectum, lungs, and breasts (Buchbinder et al., 2001).

Patients with both DM and colorectal cancer are mostly males aged over 50 years. On dermatological examination, they show specific cutaneous necrosis and ulcers, including characteristic heliotrope erythema and Gottron papules (Fig. 9) (Dourmishev and Draganov, 2009).

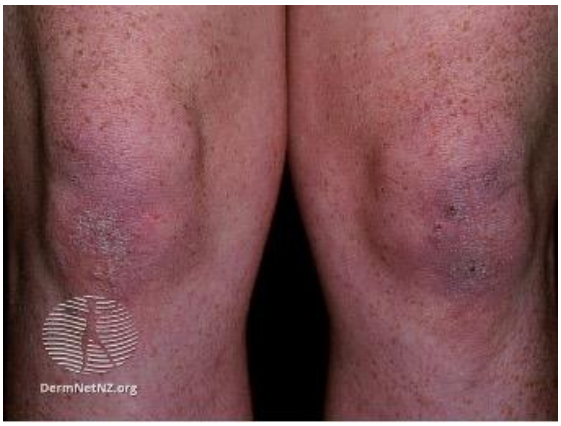


**Figure 9.** Gottron sign of dermatomyositis over the knee (DermNet, 2020).

The malignancy may precede, come concurrently with, or follow the onset of myositis symptoms (Bogdanov et al., 2018).

One of the theories that explains this correlation is quite straightforward. The immunosuppressive drugs used to treat dermatomyositis weaken the immune system, thus allowing cancer to proliferate (Szekanecz et al., 2006).

According to another hypothesis, autoimmune myositis is a paraneoplastic disease that appears after the primary cancer has developed (Dourmishev and Draganov, 2009). It assumes an immunogenic similarity between the developing malignancy and regenerating myoblasts, which provokes cross-reactivity in patients prone to autoimmunity (Casciola-Rosen et al., 2005). Among

reported studies, there is an interesting case of an 81-year-old Japanese man who complained about muscle weakness and skin eruptions. His colonoscopy revealed a stage 2 tumor which was later resected. Fourteen days after the surgery, all eruptions on the patient's skin disappeared and he was able to stand not being assisted. Sudden exacerbation of dermatomyositis symptoms post-resection is a clear indicator of possible cancer relapse (Ono et al., 2017).

There have already been many cancer screening protocols suggested, however, none of them has accurately balanced the advantages, disadvantages and the costs. Until effective guidelines are established, clinicians providing care to DM patients should promote a shared decision-making process that aligns with both the doctor's and patient's preferences (Khanna et al., 2021).

### CRONKHITE-CANADA SYNDROME

Cronkhite-Canada syndrome (CCS) is a rare acquired non-familial polyposis disease with an unknown etiology. It is characterized by numerous GI polyps and various ectodermal changes, which include alopecia, skin pigmentation changes, and nail atrophy. (Fig. 10) (Wang et al., 2017).

Watanabe et al. found the disease to be slightly more male-dominated, with symptoms arising at an average age of 63.5 years. So far, it has not been confirmed that CCS has a genetic background, however, more and more specialists notice hereditary tendencies. Another possible etiology of CCS is related to an allergic response with significantly increased IgE levels, which explains the beneficial effect of glucocorticoids (Wu et al., 2020).

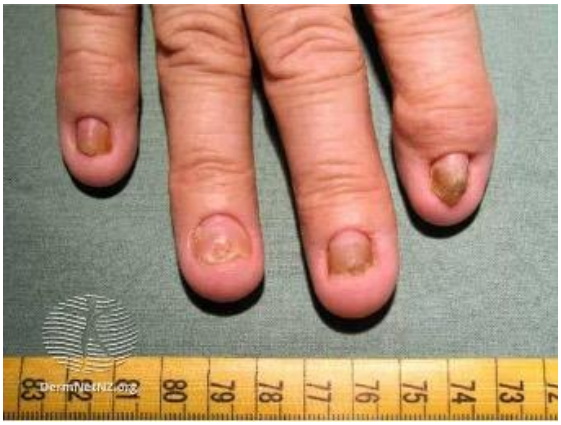


**Figure 10.** Onychodystrophy in Cronkhite-Canada syndrome (DermNet, 2021b).

Patients present with characteristic dermatological complaints and nonspecific GI symptoms like watery diarrhea, abdominal pain, and loss of appetite. CCS diagnosis should be based on the presence of polypoid lesions confirmed by endoscopy, general GI symptoms, and characteristic dermatological lesions, all combined together (Kunishi et al., 2021).

Polyps detected in CCS are of numerous types including inflammatory, hyperplastic, adenomatous, and hamartomatous polyps, that spread all over the GI tract (Wu et al., 2020). Histologically they have engorged mucosa, expanded crypt, and infiltrated lamina propria with inflammatory cells dominated by eosinophils. The presence of heterogeneous polyps in the colon alerts us of the major risk of tumor development (Wang et al., 2017).

Specific data on the risk of malignancy in CCS patients should be recorded, therefore they are advised to come for regular follow-ups to ensure prompt detection of cancerous lesions. Large polyps with the potential of malignant transformation need to be resected at an early stage (Wu et al., 2020).

*3.3. Bacterial infections uniquely associated with colorectal cancer*

Interestingly, there are several bacterial species that have been uniquely associated with colorectal cancer due to the favorable conditions the location of tumor offers. Among these are Clostridium septicum and Streptococcus gallolyticus (also known as S. bovis.) Even up to 80% of patients infected with S. bovis have concurrent colonic neoplasms (Chirikian et al., 2021).

Clostridium septicum is a rare opportunistic bacterium that presents with significant dermatological lesions and is frequently associated with colorectal cancer. Clostridium septicum is a gram-positive, anaerobic, spore-forming, opportunistic bacillus found in the gastrointestinal tract. It produces exotoxins and adhesins that have been proven to be pro-oncogenic factors to malignancies. These exotoxins induce DNA damage of intestinal epithelial cells, increasing inflammation, and enhancing cell proliferation.

Patients with Clostridium septicum bacteremia often have concomitant colorectal neoplasms (Corredoira et al., 2017). Through their anaerobic metabolism, colorectal tumors produce an acidic and hypoxic microenvironment, which greatly promotes the germination of clostridial spores (Nanjappa et al., 2015).

Clostridium infection mostly affects the soft tissues of the limbs and manifests on the skin severely as cellulitis, abscesses, and gas gangrene (or myonecrosis) (Fig. 11). Usually, the foot turns purple, and superficial erosions on the ankle and dorsum of the foot are observed.

Clostridium septicum infections are not so common in clinical practice. However, they are rapidly progressing and life-threatening conditions that may lead to sepsis, with a mortality rate as high as 50% (Slezak et al., 2022). Considering the high correlation with colorectal cancer, their presence can be a first paraneoplastic symptom, therefore the doctor should look for an underlying oncological disease. Diagnostic methods should obviously include colonoscopy and other imaging techniques to identify other coexisting neoplastic lesions (Nanjappa et al., 2015).

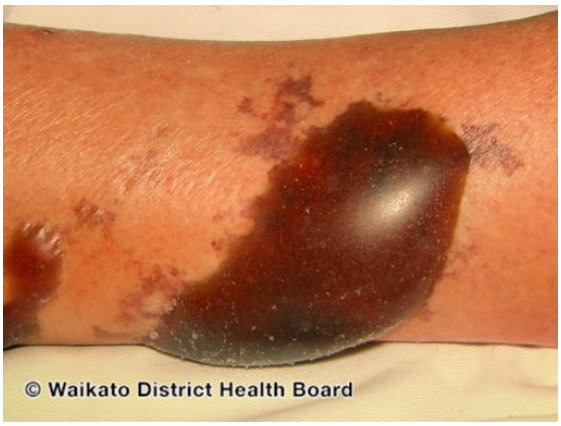


**Figure 11.** Necrotizing skin infection with gangrene (DermNet, 2016).

## 4. Discussion

Cutaneous metastases from colorectal malignancies are a rare observation, occurring in less than 5% of patients (Bittencourt et al., 2018). The presented paper discusses a wide variety of skin manifestations associated with colorectal cancer. They may precede, be concurrent with, or follow the development of the disease.

In the case of inherited diseases, such as Peutz Jeghers or Muir-Torre syndromes, skin lesions may occur before cancer develops. These genetic syndromes are manifested by characteristic symptoms, alerting the clinician to initiate screening for related types of cancer.

In paraneoplastic diseases like dermatomyositis or unique infections like Clostridium septicum infection, skin lesions may occur concurrently with cancer .

Finally, skin manifestations may appear after resection surgery, in the form of widespread metastasis or as a direct extension via surgical tracts. This is a topic of high interest to surgeons who attempt to identify the causative agents of tumor adhesion at surgical sites. The specialists need to adopt new delicate maneuvers of excision to avoid the occurrence of this iatrogenic harm.

Unlike internal symptoms, cutaneous manifestations are easily noticeable. They are disruptive to the patient's well-being, prompting them to seek medical advice quickly. Thus it is necessary for clinicians to keep an eye on new and unresolving skin lesions and remain constantly alert to potential underlying malignancies.

The authors acknowledge that the study has of a number of limitations that should be considered. The paper covers a wide range of skin manifestations linked to colorectal cancer, and although the literature search was extensive, it may be viewed as selective due to the exclusion of repetitive cases and observations with insufficient data to support the correlation. Another limitation in our research was language restriction.

The body communicates with us in extraordinary ways that we do not yet fully understand. Clinical presentation of metastasis is widely varied and poses a diagnostic challenge to clinicians. Thus, we recommend a concise investigation of skin manifestations that appear to link directly to colorectal cancer development. A condensed manual should be developed to promote early recognition and appropriate medical management.

## 5. Conclusions

Cutaneous manifestations are rare and may be a direct reflection of colorectal cancer development. Lesions may take the form of cutaneous metastasis of cancer or a paraneoplastic symptom. Correct recognition of the manifestation will enhance our screening efforts and further reduce colorectal cancer mortality rates.

## Bibliography


Aarnio M., Sankila R., Pukkala E., Salovaara R., Aaltonen L. A., De La Chapelle A., Peltomaki P., Mecklin J. P., Jarvinen H. J. 1999. Cancer Risk In Mutation Carriers Of Dna-Mismatch-Repair Genes. *International Journal of Cancer* 81**,** pp. 214–218.

Alsubait N.A., Binjadeed H.F., Alsaleh M.R., Alfaifi F.S., Alsaif F.M., Arafah M.A. 2021. Dermoscopy Of Scalp Cutaneous Metastasis Of Sigmoid Adenocarcinoma. *Jaad Case Report* 14**,** pp. 116–119.

Antonetti P., Fargnoli M.C., Porzio G., Salvatore L., Filippi R., Ghidini M., Nigro O., Gelsomino F., Zurlo I.V., Dell'aquila E., Lombardi P., Keranen S.R., Depetris I., Giampieri R., Morelli C., De Tursi M., Di Pietro F.R., Zanaletti N., Vitale P., Garajova I., Spinelli G.P., Zoratto F., Roberto M., Petrillo A., Aimar G., Cortellini A., Pensieri M.V., Ficorella C., Ferri C., Parisi, A. 2022. A Multicenter Study Of Skin Toxicity Management In Patients With Left-Sided, Ras/Braf Wild-Type Metastatic Colorectal Cancer Treated With First-Line Anti-Egfr-Based Doublet Regimen: Is There Room For Improvement? *Supportive Care in Cancer* 30**,** pp. 2455–2465.

Biller L.H., Schrag D. 2021. Diagnosis And Treatment Of Metastatic Colorectal Cancer: A Review. *Jama* 325**,** pp. 669–685.

Bittencourt M.J.S., Imbiriba A.A., Oliveira O.A., Santos J. 2018. Cutaneous Metastasis Of Colorectal Cancer. *Anais Brasileiros de Dermatologia* 93**,** pp. 884–886.

Bogdanov I., Kazandjieva J., Darlenski R., Tsankov N. 2018. Dermatomyositis: Current Concepts. *Clinics in Dermatology* 36**,** pp. 450–458.

Boland C.R., Idos G.E., Durno C., Giardiello F.M., Anderson J.C., Burke C.A., Dominitz J.A., Gross S., Gupta S., Jacobson B.C., Patel S.G., Shaukat A., Syngal S., Robertson D.J. 2022. Diagnosis And Management Of Cancer Risk In The Gastrointestinal Hamartomatous Polyposis Syndromes: Recommendations From The Us Multi-Society Task Force On Colorectal Cancer. *Gastroenterology* 162**,** pp. 2063–2085.

Buchbinder R., Forbes A., Hall S., Dennett X., Giles G. 2001. Incidence Of Malignant Disease In Biopsy-Proven Inflammatory Myopathy. A Population-Based Cohort Study. *Annals of Internal Medicine* 134**,** pp. 1087–1095.

Burris C.K.H., Rodriguez M.E., Raven M.L., Reddy D.N., Xu Y.G., Wiggs J.L., Potter H.D., Albert D.M. 2019. Muir-Torre Syndrome: The Importance Of A Detailed Family History. *Case Reports in Ophthalmology* 10**,** pp. 180–185.

Campos F.G., Figueiredo M.N., Martinez C.A. 2015. Colorectal Cancer Risk In Hamartomatous Polyposis Syndromes. *World Journal of Gastrointestinal Surg* 7**,** pp. 25–32.

Casciola-Rosen L., Nagaraju K., Plotz P., Wang K., Levine S., Gabrielson E., Corse A., Rosen, A. 2005. Enhanced Autoantigen Expression In Regenerating Muscle Cells In Idiopathic Inflammatory Myopathy. *Journal of Experimental Medicine* 201**,** pp. 591–601.

Chen H.Y., Jin X.W., Li B.R., Zhu M., Li J., Mao G.P., Zhang Y.F., Ning S. B. 2017. Cancer Risk In Patients With Peutz-Jeghers Syndrome: A Retrospective Cohort Study Of 336 Cases. *Tumour Biology* 39**,** DOI: 1010428317705131.

Chirikian D., Awsare S., Fitzgibbon J., Lee L. 2021. Concurrent Clostridium Septicum Bacteremia And Colorectal Adenocarcinoma With Metastasis To The Brain - A Case Report. *ID Cases,* 25**,** article number E01189.

Cohen P.R., Kohn S.R., Davis D.A., Kurzrock R. 1995. Muir-Torre Syndrome. *Dermatologic Clinics* 13**,** pp. 79–89.

Cohen P.R., Kohn S.R., Kurzrock R. 1991. Association Of Sebaceous Gland Tumors And Internal Malignancy: The Muir-Torre Syndrome. *The American Journal of Medicine* 90**,** pp. 606–613.

Cohn M.S., Classen R.F. 1993. The Sign Of Leser-Trelat Associated With Adenocarcinoma Of The Rectum. *Cutis* 51**,** pp. 255–257.

Constantinou C., Dancea H., Meade P. 2010. The Sign Of Leser-Trelat In Colorectal Adenocarcinoma. *The American Surgeon* 76**,** pp. 340–341.

Cooper K., Squires H., Carroll C., Papaioannou D., Booth A., Logan R.F., Maguire C., Hind D., Tappenden P. 2010. Chemoprevention Of Colorectal Cancer: Systematic Review And Economic Evaluation. *Health Technology Assessment* 14**.**
DOI: 10.3310/hta14320.

Corredoira J., Grau I., Garcia-Rodriguez J.F., Garcia-Pais M.J., Rabunal R., Ardanuy C., Garcia-Garrote F., Coira A., Alonso M.P., Boleij A., Pallares R. 2017. Colorectal Neoplasm In Cases Of Clostridium Septicum And Streptococcus Gallolyticus Subsp. Gallolyticus Bacteraemia. *European Journal of Internal Medicine* 41**,** pp. 68–73.

DermNet 2004. Available online: https://dermnetnz.org/topics/sign-of-leser-trelat (18.12.2024).

DermNet 2005. Available online: https://dermnetnz.org/imagedetail/8923-cutaneous-metastasis (18.12.2024).

DermNet 2016. Available online: https://dermnetnz.org/topics/wet-gangrene (18.12.2024).

DermNet 2020. Available online: https://dermnetnz.org/topics/adult-onset-dermatomyositis (18.12.2024).

DermNet 2021a. Available online: https://dermnetnz.org/topics/lynch-syndrome (18.12.2024).

DermNet 2021b. Available online: https://dermnetnz.org/topics/cronkhite-canada-syndrome (18.12.2024).

DermNet 2022a. Available online: https://dermnetnz.org/topics/cowden-disease (18.12.2024).

DermNet 2022b. Available onlne: https://dermnetnz.org/topics/tripe-palms (18.12.2024).

DermNet 2023. Available online: https://dermnetnz.org/images/elephantiasis-nostras--verrucosa-images (18.12.2024).

Dourmishev L.A., Draganov P.V. 2009. Paraneoplastic Dermatological Manifestation Of Gastrointestinal Malignancies. *World Journal of Gastroenterology* 15**,** pp. 4372–4379.

Durak D., Alkurt E.G., Turhan V.B., Tutan B., Sahiner I.T., Kendirci M. 2022. Comparison Of Short-Term Results Of Laparoscopic And Open Surgeries For Colorectal Cancer: A Single-Center Experience. *The Cureus Journal of Medical Science* 14**,** article number E24635.

Ellis D.L., Kafka S.P., Chow J.C., Nanney L.B., Inman W.H., Mccadden M.E., King L.E. Jr. 1987. Melanoma, Growth Factors, Acanthosis Nigricans, The Sign Of Leser-Trelat, And Multiple Acrochordons. A Possible Role For Alpha-Transforming Growth Factor In Cutaneous Paraneoplastic Syndromes. *The New England Journal of Medicine* 317**,** pp. 1582–1587.

Erkek E., Hizel S., Sanly C., Erkek A.B., Tombakoglu M., Bozdogan O., Ulkatan S., Akarsu C. 2005. Clinical And Histopathological Findings In Bannayan-Riley-Ruvalcaba Syndrome. *Journal of the American Academy of Dermatology* 53**,** pp. 639–643.

Ferlay J., Soerjomataram I., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D.M., Forman D., Bray F. 2015. Cancer Incidence And Mortality Worldwide: Sources, Methods And Major Patterns In Globocan 2012. *International Journal of Cancer* 136**,** pp. E359–E386.

Ginarte M., Sanchez-Aguilar D., Toribio J. 2001. Sign Of Leser-Trelat Associated With Adenocarcinoma Of The Rectum. *European Journal of Dermatology* 11**,** pp. 251–253.

Gkegkes I.D., Minis E.E., Iavazzo C. 2018. Dermatomyositis And Colorectal Cancer: A Systematic Review. *Irish Journal of Medical Science* 187**,** pp. 615–620.

Gunduz K., Coban M., Ozturk F., Ermertcan A.T. 2013. Malignant Acanthosis Nigricans Associated With Ileocecal Adenocarcinoma. *Cutaneous and Ocular Toxicology* 32**,** pp. 173–175.

Hakami, R., Alali, M. N., Alshammari, T., Alshammari, S., Alyahya, Z., Ayesh, M., Alsaad, K. & Abduljabbar, A. 2020. A Cutaneous Metastasis Of Unresectable Rectal Adenocarcinoma: A Case Report And Literature Review. *Int J Surg Case Rep,* 71**,** 95-101.

Heald B., Mester J., Rybicki L., Orloff M.S., Burke C.A., Eng C. 2010. Frequent Gastrointestinal Polyps And Colorectal Adenocarcinomas In A Prospective Series Of Pten Mutation Carriers. *Gastroenterology* 139**,** pp. 1927–1933.

Heaphy M.R. Jr., Millns J.L., Schroeter A.L. 2000. The Sign Of Leser-Trelat In A Case Of Adenocarcinoma Of The Lung. *Journal of the American Academy of Dermatology* 43**,** pp. 386–390.

Hemminki A., Avizienyte E., Roth S., Loukola A., Aaltonen L.A., Jarvinen H., De La Chapelle A. 1998. [A Serine/Threonine Kinase Gene Defective In Peutz-Jeghers Syndrome]. *Duodecim* 114**,** pp. 667–668.

Heng M.C., Soo-Hoo K., Levine S., Petresek D. 1988. Linear Seborrheic Keratoses Associated With Underlying Malignancy. *Journal of the American Academy of Dermatology* 18**,** pp. 1316–1321.

Holdiness M.R. 1987. A Case Of Leser-Trelat Sign. *Journal of the American Academy of Dermatology* 16**,** pp. 147–148.

Kant P., Hull M.A. 2011. Excess Body Weight And Obesity--The Link With Gastrointestinal And Hepatobiliary Cancer. *Nature Reviews Gastroenterology & Hepatology* 8**,** pp. 224–238.

Kemal Y., Odabasi E.A., Kemal O., Bakirtas M. 2018. Cutaneous Metastasis Of Colon Adenocarcinoma. *Turkish Journal of Surgery* 34**,** pp. 237–239.

Khanna U., Galimberti F., Li Y., Fernandez A.P. 2021. Dermatomyositis And Malignancy: Should All Patients With Dermatomyositis Undergo Malignancy Screening? *Annals of Translational Medicine* 9**,** article number 432.

Kijima S., Sasaki T., Nagata K., Utano K., Lefor A.T., Sugimoto H. 2014. Preoperative Evaluation Of Colorectal Cancer Using Ct Colonography, Mri, And Pet/Ct. *World Journal of Gastroenterology* 20**,** pp. 16964–16975.

Kudibal M.T., Venzo A. 2018. [Muir-Torre Syndrome]. *Ugeskrift for Læger,* 180, p. 2–3.

Kunishi Y., Yoshie K., Kato Y., Maeda S. 2021. Polypoid Lesions In The Stomach And The Colon: Cronkhite-Canada Syndrome. *The Lancet Gastroenterology and Hepatology* 6**,** article number 770.

Lazar, A. J., Lyle, S. & Calonje, E. 2007. Sebaceous Neoplasia And Torre-Muir Syndrome. *Curr Diagn Pathol,* 13**,** 301-319.

Lee S.S., Jung N.J., Im M., Lee Y., Seo Y.J., Lee J. H. 2011. Acral-Type Malignant Acanthosis Nigricans Associated With Gastric Adenocarcinoma. *Annals of Dermatology Annals of Dermatology* 23**,** pp. S208–S210.

Lim A., Ngeow J. 2021. The Skin In Cowden Syndrome. *Frontiers in Medicine* 8**,** article number 658842.

Malla S., Razik A., Vyas S. 2019. Cutaneous Metastasis In Adenocarcinoma Rectum. *BMJ Case Reports* 12. DOI: 10.1136/bcr-2019-229652.

Mathiak M., Rutten A., Mangold E., Fischer H.P., Ruzicka T., Friedl W., Propping P., Kruse R. 2002. Loss Of Dna Mismatch Repair Proteins In Skin Tumors From Patients With Muir-Torre Syndrome And Msh2 Or Mlh1 Germline Mutations: Establishment Of Immunohistochemical Analysis As A Screening Test. *The American Journal of Surgical Pathology* 26**,** pp. 338–343.

Miyahara K., Tobe S., Shizuku T., Inamoto R., Katayama I. 2020. Colon Cancer Of Peutz-Jeghers Syndrome With Gallolyticus Endocarditis. *Clinical Journal of Gastroenterology* 13**,** pp. 517–521.

Nanjappa S., Shah S., Pabbathi S. 2015. Clostridium Septicum Gas Gangrene In Colon Cancer: Importance Of Early Diagnosis. *Case Reports in Infectious Diseases* 2015**,** article number 694247.

Nesseris I., Tsamakis C., Gregoriou S., Ditsos I., Christofidou E., Rigopoulos D. 2013. Cutaneous Metastasis Of Colon Adenocarcinoma: Case Report And Review Of The Literature. *Anais Brasileiros de Dermatologia* 88**,** pp. 56–58.

Oh B.Y., Lee R.A., Chung S.S., Kim K.H. 2011. Epidermal Growth Factor Receptor Mutations In Colorectal Cancer Patients. *Journal of the Korean Society of Coloproctology* 27**,** pp. 127–132.

Okuno S., Hashimoto T., Matsuo S., Satoh T. 2022. Erosive Pustular Dermatosis Of The Scalp-Like Eruption From Panitumumab. *Australasian Journal of Dermatology* 63**,** pp. 271–272.

Ono K., Shimomura M., Toyota K., Kagimoto A., Tsukiyama N., Shishida M., Oishi K., Miyamoto K., Shibata S., Ikeda M., Sadamoto S., Takahashi T. 2017. Successful Resection Of Liver Metastasis Detected By Exacerbation Of Skin Symptom In A Patient With Dermatomyositis Accompanied By Rectal Cancer: A Case Report And Literature Review. *Surgical Case Reports* 3**,** article number 3.

Pasquereau-Kotula E., Martins M., Aymeric L., Dramsi S. 2018. Significance Of Streptococcus Gallolyticus Subsp. Gallolyticus Association With Colorectal Cancer. *Frontiers in Microbiology* 9**,** article number 614.

Patel S.G., Karlitz J.J., Yen T., Lieu C.H., Boland C.R. 2022. The Rising Tide Of Early-Onset Colorectal Cancer: A Comprehensive Review Of Epidemiology, Clinical Features, Biology, Risk Factors, Prevention, And Early Detection. *The Lancet Gastroenterology and Hepatology* 7**,** pp. 262–274.

Pettey A.A., Walsh J.S. 2005. Muir-Torre Syndrome: A Case Report And Review Of The Literature. *Cutis* 75**,** pp. 149–155.

Phiske M.M. 2014. An Approach To Acanthosis Nigricans. *Indian Dermatology Online Journal* 5**,** pp. 239–249.

Pilarski R., Burt R., Kohlman W., Pho L., Shannon K.M., Swisher E. 2013. Cowden Syndrome And The Pten Hamartoma Tumor Syndrome: Systematic Review And Revised Diagnostic Criteria. *JNCI: Journal of the National Cancer Institute* 105**,** pp. 1607–1616.

Ponti G., Luppi G., Losi L., Giannetti A., Seidenari S. 2010. Leser-Trelat Syndrome In Patients Affected By Six Multiple Metachronous Primitive Cancers. *Journal of Hematology & Oncology* 3**,** article number 2.

Ponti G., Ponz De Leon M. 2005. Muir-Torre Syndrome. *The Lancet Oncology* 6**,** pp. 980–987.

Rajan D., Shah M., Raghavan P., Mujeeb S., Rashid S., Desouza A., Mustacchia P. 2012. Lower Extremity Cutaneous Lesions As The Initial Presentation Of Metastatic Adenocarcinoma Of The Colon. *Case Reports in Medicine* 2012**,** article number 989104.

Rampen H.J., Schwengle L.E. 1989. The Sign Of Leser-Trelat: Does It Exist? *Journal of the American Academy of Dermatology* 21**,** pp. 50–55.

Sadler K.A., Baxter M.A., Peters A.L., Grose D. 2020. Metastatic Cutaneous Deposits As The Initial Feature Of Rectal Adenocarcinoma. *Scottish Medical Journal* 65**,** pp. 28–31.

Schwartz R.A., Helmold M.E., Janniger C.K., Gascon P. 1991. Sign Of Leser-Trelat With A Metastatic Mucinous Adenocarcinoma. *Cutis* 47**,** pp. 258–260.

Schwartz R.A., Torre D.P. 1995. The Muir-Torre Syndrome: A 25-Year Retrospect. *Journal of the American Academy of Dermatology* 33**,** pp. 90–04.

Shah M., Shah N.B., Moder K.G., Dean D. 2013. Three Cases Of Dermatomyositis Associated With Papillary Thyroid Cancer. *Endocrine Practice* 19**,** pp. E154–E157.

Shakil S., Aldaher Z., Divalentin L. 2022. Peutz-Jeghers Syndrome Presenting With Anemia: A Case Report. *The Cureus Journal of Medical Science* 14**,** article number E26481.

Simic D., Dummer R., Freiberger S.N., Ramelyte E., Barysch M.J. 2021. Clinical And Molecular Features Of Skin Malignancies In Muir-Torre Syndrome. *Genes* 12, article number 781.

Slezak M., Smolar M., Drobna Saniova B., Hosala M., Miklusica J. 2022. Clostridium Septicum Foot Gangrene Associated With Colorectal Cancer. *Neuro Enocrinology Letters* 43**,** pp.57–64.

Smerdel M.P., Skytte A.B., Jelsig A.M., Ebbehoj E., Stochholm K. 2020. Revised Danish Guidelines For The Cancer Surveillance Of Patients With Cowden Syndrome. *European Journal of Medical Genetics* 63**,** article number 103873.

Stanich P.P., Owens V.L., Sweetser S., Khambatta S., Smyrk T.C., Richardson R.L., Goetz M.P., Patnaik M.M. 2011. Colonic Polyposis And Neoplasia In Cowden Syndrome. *Mayo Clinic Proceedings* 86**,** pp. 489–492.

Strate L.L., Syngal S. 2005. Hereditary Colorectal Cancer Syndromes. *Cancer Causes & Control* 16**,** pp. 201–213.

Szekanecz E., Andras C., Sandor Z., Antal-Szalmas P., Szanto J., Tamasi L., Kiss E., Szekanecz Z. 2006. Malignancies And Soluble Tumor Antigens In Rheumatic Diseases. *Autoimmunity Reviews* 6**,** pp. 42–47.

Tan M.H., Mester J.L., Ngeow J., Rybicki L.A., Orloff M.S., Eng C. 2012. Lifetime Cancer Risks In Individuals With Germline Pten Mutations. *Clinical Cancer Research* 18**,** pp. 400–407.

Theodoropoulos G.E., Karafoka E., Papailiou J.G., Stamopoulos P., Zambirinis C.P., Bramis K., Panoussopoulos S.G., Leandros E., Bramis J. 2009. P53 And Egfr Expression In Colorectal Cancer: A Reappraisal Of 'Old' Tissue Markers In Patients With Long Follow-Up. *Anticancer Research* 29**,** pp. 785–791.

Tsutsui K., Namikawa K., Mori T., Kato K., Jinnai S., Nakama K., Ogata D., Takahashi A., Yamazaki N. 2021. Case Of Acquired Reactive Perforating Collagenosis Induced By Panitumumab For Colon Cancer. *The Journal of Dermatology* 48**,** pp. E114–E115.

Van Lier M.G., Mathus-Vliegen E.M., Wagner A., Van Leerdam M.E., Kuipers E.J. 2011. High Cumulative Risk Of Intussusception In Patients With Peutz-Jeghers Syndrome: Time To Update Surveillance Guidelines? *American Journal of Gastroenterology* 106**,** pp. 940–945.

Wang J., Zhao L., Ma N., Che J., Li H., Cao B. 2017. Cronkhite-Canada Syndrome Associated With Colon Cancer Metastatic To Liver: A Case Report. *Medicine* 96**,** article number E7466.

Weiss J.M., Gupta S., Burke C.A., Axell L., Chen L.M., Chung D.C., Clayback K.M., Dallas S., Felder S., Gbolahan O., Giardiello F.M., Grady W., Hall M.J., Hampel H., Hodan R., Idos G., Kanth P., Katona B., Lamps L., Llor X., Lynch P.M., Markowitz A.J., Pirzadeh-Miller S., Samadder N.J., Shibata D., Swanson B.J., Szymaniak B.M., Wiesner G.L., Wolf A., Yurgelun M.B., Zakhour M., Darlow S.D., Dwyer M.A., Campbell M. 2021. Nccn Guidelines(R) Insights: Genetic/Familial High-Risk Assessment: Colorectal, Version 1.2021. *Journal of the National Comprehensive Cancer Network* 19**,** pp. 1122–1132.

Wu Z.Y., Sang L.X.. Chang B. 2020. Cronkhite-Canada Syndrome: From Clinical Features To Treatment. *Gastroenterology Report* 8**,** pp. 333–342.

Yang Y., Chen R., Zhang R. 2020. Cutaneous Metastasis Of A Colon Adenocarcinoma Presenting As An Unusual Manifestation: A Report Of One Case. *International Journal of Clinical and Experimental Pathology* 13**,** pp. 1897–1901.

UMEDICAL REPORTS

# LONG-TERM DOPPLER ULTRASOUND MONITORING OF PATIENTS WITH SYMPTOMATIC AND ASYMPTOMATIC STENOSIS OF THE CAROTID ARTERIES WITH EVALUATION OF DYNAMICS OF THE ATHEROSCLEROTIC PROCESS

## DŁUGOTERMINOWA OBSERWACJA ZA POMOCĄ USG DOPPLER PACJENTÓW Z OBJAWOWYM I NIEOBJAWOWYM ZWĘŻENIEM TĘTNIC SZYJNYCH, Z OCENĄ DYNAMIKI PROCESU MIAŻDŻYCY

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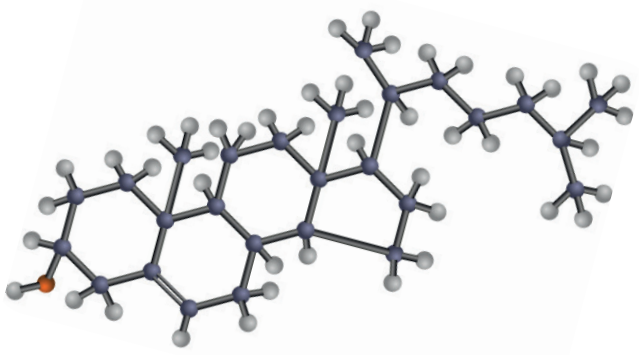

**Abstract:** Macroangiopathy is one of the most important risk factors/causes of ischemic, non-cardioembolic stroke. Atherosclerotic stenosis of the common carotid artery/internal carotid artery (CCA/ICA) is considered more dangerous when plaque presents as hypoechogenic in USG imaging. The main aim of the study was prospective observation/monitoring of changes of the condition of carotid arteries (CCA and ICA) in patients with symptomatic and asymptomatic carotid stenosis. The more specific goals included evaluation of the degree of arterial stenosis, morphology of atherosclerotic plaques, intima-media complex (IMC) thickness, and biochemical stroke/cardiovascular risk factors.

The study included 48 patients with at least 50% carotid stenosis, with a mean age of 66.75 years when entering the study. Twenty four patients (50%) who had previously been hospitalized due to ischemic stroke/transient ischemic attack (TIA) were classified as symptomatic patients. Ten healthy control subjects with a mean age of 67.7 years had never shown symptoms of stroke or USG evidence of carotid stenosis. Both groups underwent long-term clinical observation covering a period between one and a half and three years. Subsequent visits and examinations, including Doppler USG evaluation of the carotid and vertebral arteries, were performed every four months (in the study group) or every eight months (in the healthy controls). Statistically analyzed data were collected for over two years in the study group and one and a half years in the controls.
Throughout a 16-month period of monitoring the condition of carotid arteries, we observed progression of arterial stenosis, which was most prominent over the first eight months (Visits 1-4), and less pronounced in the following nine months (Visits 5-8), with a slight regression at the end of the observation period. A prospective analysis of plaque structure showed significant changes in its morphology, developing over the whole period of the study. Plaques classified as unstable (hypoechogenic) at the beginning of our study tended to transform into stable ones (hyperechogenic) with time (except for the last four months).

The assessed changes in intima-media thickness were not found to be statistically significant over the whole study period. Among all the laboratory tests (blood morphology, hematology, serum lipids, fasting blood glucose level, glucose loading test, HbA1c, sedimentation rate, and C-reactive protein), only total cholesterol levels changed considerably over the period of the 20-month observation. Among all the risk factors studied at the same time-points as Doppler ultrasound was performed, only one parameter, i.e. total cholesterol level, showed statistically significant differences between the groups. There were no changes in any of the parameters assessed in the control group.

The study findings prove that, if performed every four months in both symptomatic and asymptomatic patients with at least 50% carotid artery stenosis, Doppler USG examination seems to be a reasonable method for monitoring the dynamics of atherosclerotic processes which lead to narrowing of the arterial lumen, deleterious transformation of the intima-media complex and changes in the morphology of carotid atherosclerotic plaques. Therefore, it may be a useful tool and paradigm of examination which has a considerable preventive value.

**Key words**: ischemic stroke, cerebrovascular disease, carotid stenosis, atherosclerotic plaques, IMC, IMT, Doppler USG, cholesterol

**Streszczenie:** Makroangiopatia jest jednym z najważniejszych czynników ryzyka/powodów niedokrwiennego, niesercopochodnego udaru mózgu. Miażdżycowe zwężenie tętnicy szyjnej wspólnej/tętnicy szyjnej wewnętrznej (CCA/ICA) jest uważane za tym groźniejsze, im bardziej hypoechogeniczna jest blaszka miażdżycowa w badaniu USG. Celem tego badania była prospektywna obserwacja / monitorowanie zmian stanu tętnic szyjnych (CCA i ICA) u pacjentów z objawowym i nieobjawowym zwężeniem tych tętnic. Bardziej szczegółowe cele były związane z oceną stopnia stenozy tętnic szyjnych, morfologii blaszek miażdżycowych, grubości kompleksu intima-media (IMC) oraz biochemicznych czynników ryzyka udaru mózgu / chorób sercowo-naczyniowych.

Badanie było przeprowadzone na 48 pacjentach z co najmniej 50-proc. zwężeniem tętnicy szyjnej. Średni wiek w tej grupie wynosił 66,75 lat w chwili przystępowania do badania. Dwudziestu pięciu pacjentów (50%), którzy wcześniej byli hospitalizowani z powodu niedokrwiennego udaru mózgu / TIA, sklasyfikowano jaki pacjentów objawowych. Grupę kontrolną stanowiło 10 zdrowych osób o średniej wieku 67,7 lat, które nigdy nie miały objawów udaru mózgu ani danych na zwężenie tętnicy szyjnej w badaniu USG. Obie grupy pacjentów podlegały długoterminowej obserwacji klinicznej (1,5–3 lat), zaś kolejne konsultacje i badania, w tym badanie USG Doppler tętnic szyjnych i kręgowych, były wykonywane co 4 miesiące (w grupie ze stenozą) lub co 8 miesięcy (w grupie kontrolnej). Analiza statystyczna była przeprowadzona na danych uzyskanych w ciągu 2 lat obserwacji grupy ze zwężeniem tętnicy, oraz 1,5 roku obserwacji grupy kontrolnej.

W ciągu 16-miesięcznego monitorowania stanu tętnic szyjnych obserwowaliśmy postęp stenozy tych tętnic, który był najsilniej wyrażony przez pierwsze 8 miesięcy badania (między wizytami 1–4), a mniej wyrażona w ciągu kolejnych 8 miesięcy obserwacji (między wizytami 5–8), z niewielkim cofaniem się zwężenia pod koniec okresu obserwacji. Prospektywna analiza struktury blaszek miażdżycowych wykazała znamienne różnice w morfologii blaszek w czasie trwania całego badania. Blaszki zakwalifikowane na początku badania jako niestabilne (hipoechogeniczne) miały tendencję do przekształcania się w blaszki stabilne (hiperechogeniczne), z wyjątkiem ostatnich 4 miesięcy. Ocena zmian grubości kompleksu intima-media w trakcie trwania badania nie wykazała statystycznie znamiennych różnic. Spośród wszystkich wykonanych testów laboratoryjnych (morfologia krwi, hematologia, profil lipidowy, glikemia na czczo, krzywa obciążenia glukozą, HbA1c, OB., CRP) tylko wartości stężenia cholesterolu całkowitego we krwi wykazały znamienne różnice w odstępie 20-miesięcznej obserwacji. W grupie kontrolnej nie było żadnych zmian w żadnym z badanych parametrów.

Podsumowując wyniki badania, ocena co 4 miesiące za pomocą USG Doppler stanu zwężenia tętnicy szyjnej u pacjentów objawowych lub nie objawowych, ale mających co najmniej 50-proc. stenozę tętnicy szyjnej, wydaje się racjonalnym sposobem monitorowania dynamiki procesu miażdżycy, który prowadzi do zwężania się tętnic, szkodliwej transformacji kompleksu intima-media, jak również takich niekorzystnych zmian morfologii blaszek miażdżycowych w tętnicach szyjnych i mogłaby być użytecznym narzędziem i paradygmatem badania mającym „prewencyjne" zalety w profilaktyce niedokrwiennego udaru mózgu.

**Słowa kluczowe:** udar niedokrwienny mózgu, choroba sercowo-naczyniowa, zwężenie tętnicy szyjnej, blaszka miażdżycowa, IMC, IMT, USG Doppler, cholesterol

**Acronyms**

AF – atrial fibrillation
CAS – carotid angioplasty with stent
CCA – common carotid artery
CEA – carotid endarterectomy
ICA – internal carotid artery
ICF – informed consent form
IMC – intima-media complex
SD – standard deviation
TGA – transient global amnesia
TIA – transient ischemic attack
Doppler USG – Doppler ultrasonography
VA – vertebral artery

## Introduction

Atherosclerosis of the vessels supplying blood to the brain is one of the key conditions underlying ischemic stroke. Among them is macroangiopathy, i.e. an atherosclerotic process affecting the carotid arteries and one of the major risk factors/causes of ischemic, non-cardioembolic stroke. Atherosclerotic stenosis of the common carotid artery (CCA) and the internal carotid artery (ICA) is considered more dangerous when plaque presents as hypoechogenic in ultrasound imaging. More dangerous plaque is associated with both larger lipidic deposits and lower calcification at the same time (Abd-Alla et al., 2012; Garvey et al. 2000; Rothwell et al., 2003). Antiplatelets (acetylsalicylic acid, clopidogrel) are suitable and sufficient antithrombotic preventives against ischemic stroke in patients with mild and moderate stenosis, while in some individuals, endarterectomy or angioplasty with stent, as a more effective method of secondary prevention, especially in the case of stroke/TIA, are obligatory. However, it is not only the degree of stenosis (70-99%) that should be considered but also hemodynamic conditions. Although CT angiography of the carotid arteries is a precise method of evaluation of stenosis degree, it does not allow to measure velocity of the blood flow or assess structure of the arterial wall - intima-media thickness (IMT) or morphology of atherosclerotic plaques in terms of their content and stability. Doppler ultrasound imaging is a noninvasive and accurate method of detecting all of these parameters (Masayuki et al., 2024, Matos et al., 2014).

## Material and Methods

The study was approved by the Ethics Committee of the Medical University of Lodz.

PATIENTS

The study was performed in 48 patients (17 females and 31 males) with at least 50% carotid stenosis (in some cases bilateral) with a mean age of 66.75 years (standard deviation 9.4 years), who gave informed consent. Twenty-four individuals (50%) had previously been hospitalized due to ischemic stroke/TIA and were classified as symptomatic patients.

Ten healthy controls (six females and four males), with a mean age of 67.7 years (standard deviation 8.5 years), showed no symptoms of stroke or ultrasound evidence of carotid stenosis when entering the study.

There were no statistical differences in the mean age of the groups ($p>0.05$) or in terms of concomitant diseases (Table 1).

**Table 1**. Baseline characteristics of the patients in the study and control groups.

| No. | Disease | Study group (N=48) | Percentage of patients | Control group (N=10) | Percentage of patients | Statistical evaluation |
|---|---|---|---|---|---|---|
| 1 | Hypertensive disease | 34 | 70.83 | 4 | 40.00 | **Yates's chi-squared test =2.25 p>0.05** |
| 2 | Diabetes | 6 | 12.50 | 3 | 30.00 | **Yates's chi-squared test =0.83 p>0.05** |
| 3 | Atrial fibrillation | 9 | 18.75 | 3 | 30.00 | **Yates's chi-squared test =0.14 p>0.05** |

**Table 1**. Baseline characteristics of the patients in the study and control groups (cont.)

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | Coronary heart disease | 11 | 22.92 | 1 | 10.00 | **Fisher's exact test p>0.05** |
| 5 | Myocardial infarction (past) | 10 | 20.83 | 2 | 20.00 | **Fisher's exact test p>0.05** |
| 6 | Stroke (past) | 25 | 52.08 | 0 | 0.00 | **Fisher's exact test p<0.01** |
| 7 | No other diseases | 0 | 0.00 | 2 | 20.00 | **Fisher's exact test p<0.05** |
| 8 | Smoking | 11 | 22.92 | 2 | 20.00 | **Fisher's exact test p>0.05** |

### INCLUSION AND EXCLUSION CRITERIA

- Inclusion criteria: signed informed consent form (ICF), presence of plaque in at least one of the carotid arteries with a hemodynamic effect (for the study group), ability to visit hospital for medical examination every four months for a total period of three years.
- Exclusion criteria: withdrawal of consent, physical or mental disability affecting scheduled visits.

Both groups underwent a long-term clinical and diagnostic observation covering a period between one and a half and three years.

The statistically analyzed data were collected over a minimum period of two years in the study group and one and a half years in the controls.

### METHODS

The study group and the control subjects underwent Doppler USG examinations (every four or eight months, respectively) performed with the use of the Siemens Acuson CV70 system. The arterial lumen, blood flow, IMT and plaque structure were measured at midday hours and analyzed. Software dedicated for the carotid and vertebral arteries and a 10MHz head were applied. The parameters of CCA, ICA and the vertebral artery (VA) were examined bilaterally.

Due to different schedules of appointments (every eight months for the healthy volunteers and every four months for the study group), Visit 2 for the former and Visit 3 for the latter were held in the same time point. Similarly, Visits 5 and 3 for the study group and Visits 3 and 2 for the control group were equivalent for both groups due to the same schedule.

Stenosis was estimated based on the European Carotid Surgery Trial (ECST). As a complementary procedure, the following converter based on the blood flow was used (Table 2).

**Table 2**. Method of conversion of abnormal blood flow into percentage of arterial stenosis.

| Stenosis | PSV [cm/s] | EDV [cm/s] | Turbulences | ICA/CCA | ICA/CCA above |
|---|---|---|---|---|---|
| 0-30% | <125 | <40 | - | <1.8 | 0.8-1.0 |
| 30-40% | <125 | <40 | -/+ | <1.8 | 0.8-1.0 |
| 40-50% | 125 | 40 | + | 2.0 | 1.2-1,5 |
| 50-60% | <150 | 50 | + | <3.0 | 1.2-2.0 |
| 60-70% | >200 | <100 | ++ | 4.5 | 2.0-3.0 |
| >70% | >250 | >100 | +++ | >5.0 | >4 |
| >90% | >300 | >140 | +++ | >5.0 | >8 |
| >95% | <100 | <40 | +/- | <1.8 | 1.0-3.0 |

In specific cases, additional modifications were applied:

- stenosis >7_0% or coexisting contralateral CCA/ICA occlusion **– 10%**
- short-length plaque – **10%**
- extended multilevel stenosis **+ 15%**
- significant flow acceleration in VA **+ 15%**.

For the statistic purposes, all plaques were classified either as stable or unstable based on the Bluth method (Bluth EI et al. 1986).

The IMT was measured 1 cm below the sinus (lower variability in the CCA than the ICA) in both frontal and lateral projections. The mean was evaluated accordingly.

At the same time-points as USG-D examinations, blood samples were taken from the patients for evaluation of morphology, hematology, serum lipids, fasting blood glucose level, glucose loading test, HbA1c, sedimentation rate and c-reactive protein.

The patients were asked to restrain from fatty foods and tobacco smoking throughout the whole study period.

STATISTICAL METHODS

The arithmetic mean ($\bar{x}$) median *(Me)*, standard deviation (SD) and skewness were appropriately applied. For further analysis, the following tests were performed: the Shapiro–Wilk test, the t-Student test, Yates's chi-squared test, the Mann-Whitney test, the Levene test, the Wilcoxon test, Friedman's ANOVA test and Spearman's rank correlation coefficient.
Differences were considered as statistically significant at $p \leq 0.05$.
For all the statistic calculations, the Statistica 10.1PL and MS Office software were used.

## Results

PROSPECTIVE OBSERVATION OF THE DYNAMICS OF CAROTID STENOSIS CHANGES

Over the 16-month period of monitoring the condition of carotid arteries, we observed progression of arterial stenosis, which was most prominent in the first eight months (Visits 1-4) and less pronounced in the following eight months (Visits 5-8), with little regression at the end of observation period. The exact values of stenosis (expressed as percentage %) at all the visits and a relative comparison of the subsequent visits are shown in Table 3.

Statistically significant differences in progression of carotid stenosis were observed between the following visits: 1 vs. 3, 1 vs. 4, 1 vs. 5, 2 vs. 3, 2 vs. 4, 2 vs. 5, 2 vs. 6, 3 vs. 4.

**Table 3**. Statistical analysis of the dynamics of carotid stenosis progression and relative comparison of the values between the subsequent visits.

| **Stenosis (%)** | **Study group** | | | | | |
|---|---|---|---|---|---|---|
| | **Visit 1** | **Visit 2** | **Visit 3** | **Visit 4** | **Visit 5** | **Visit 6** |
| Number of patients | 76 | 75 | 74 | 74 | 67 | 60 |
| Minimum | 25.00 | 25.00 | 30.00 | 30.00 | 30,00 | 30.00 |
| Maximum | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Median | 60.00 | 60.00 | 65.00 | 61.50 | 60.00 | 60.00 |
| Average | 62.63 | 62.77 | 65.11 | 66.14 | 65.51 | 65.05 |
| Standard deviation (SD) | 19.81 | 19.88 | 18.85 | 19.00 | 19.62 | 19.54 |
| Asymmetry index | 0.45 | 0.36 | 0.32 | 0.27 | 0.36 | 0.40 |
| Statistical evaluation | **Friedman's ANOVA Test F=21.07 p<0.001** (21.06973 p=0.00079) **Wilcoxon Z-Test $^{1,2}$ Z = 1.02 p>0.05; Wilcoxon Z-Test $^{1,3}$ Z = 3.22 p<0.01 Wilcoxon Z-Test $^{1,4}$ Z = 3.96 p<0.001; Wilcoxon Z-Test $^{1,5}$ Z = 2.22 p<0.05 Wilcoxon Z-Test $^{1,6}$ Z = 1.76 p>0.05; Wilcoxon Z-Test $^{2,3}$ Z = 3.93 p<0.001 Wilcoxon Z-Test $^{2,4}$ Z = 4.47 p<0.001; Wilcoxon Z-Test $^{2,5}$ Z = 2.37 p<0.05 Wilcoxon Z-Test $^{2,6}$ Z = 2.19 p<0.05; Wilcoxon Z-Test $^{3,4}$ Z = 1.98 p<0.05 Wilcoxon Z-Test $^{3,5}$ Z = 0.76 p>0.05; Wilcoxon Z-Test $^{3,6}$ Z = 0.50 p>0.05 Wilcoxon Z-Test $^{4,5}$ Z = 0.01 p>0.05; Wilcoxon Z-Test $^{4,6}$ Z = 0.08 p>0.05 Wilcoxon Z-Test $^{5,6}$ Z = 0.38 p>0.05** | | | | | |

The dynamics of carotid stenosis progression observed over six visits held in the period of 20 months is presented in Figure 1.

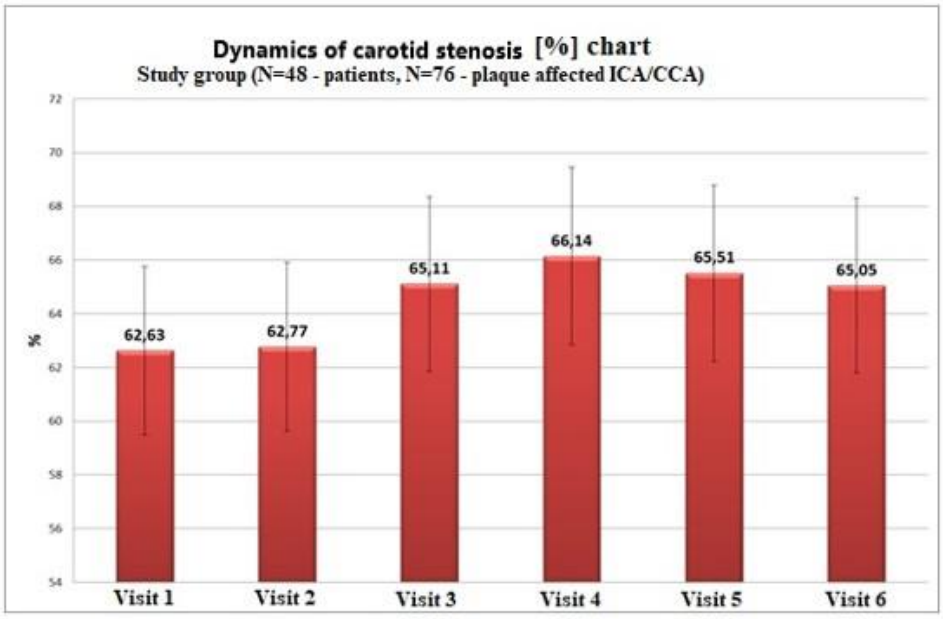


**Figure 1.** Dynamics of carotid stenosis monitored over 20 months.

Evaluation of stenosis progression vs. no progression in the study and control groups over the first eight months (based on comparison between Visits 3-1 in the stenotic patients and Visits 2-1 in the controls) revealed a significant difference between previously measured values of these two groups (p=0.0001) (Figure 2). The same result was noted in the following eight months of the observation period (Visits 5-3 for the study group and Visits 3-2 for the control subjects) (p=0.00008) (Figure 3).

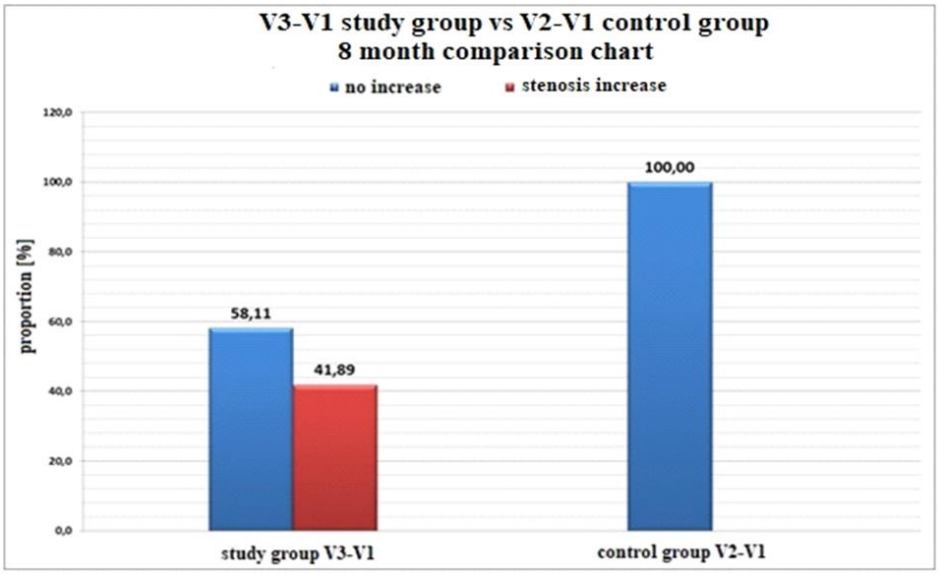


**Figure 2**. Proportion of the patients who showed progression of carotid stenosis over the first eight months of the study. The difference in progression of carotid stenosis in the study group vs. the control group was statistically significant (p=0.0001).

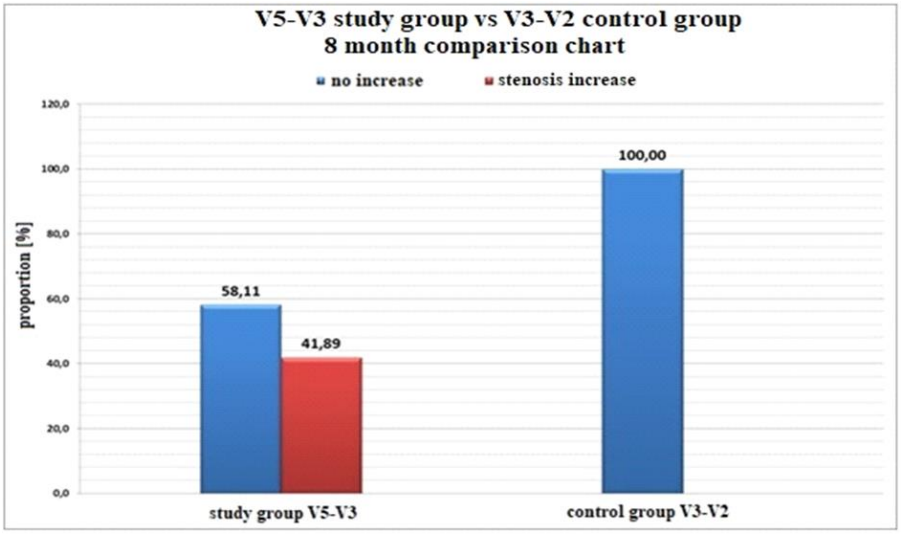


**Figure 3**. Proportion of the patients who showed progression of carotid stenosis over the next eight months of the study. The difference in progression of carotid stenosis in the study group vs. control group was statistically significant (p=0.00008).

We noticed bidirectional changes in the condition of carotid stenosis in the study subjects, however, over the first visits they were consistently progressive. In the first year of observation (Visits 1-4) we recorded an increasing stenosis, whereas in the second year (Visits 4-6) stenosis slightly decreased. Statistically significant changes were observed between Visit 1 and Visit 5. At that time, all the patients received 40 mg of simvastatin or 80 mg of atorvastatin a day. Statistical significance of arterial lumen changes ranged between $p< 0.000001$ and $p<0.001$. It applied both to the long-term observation assessment and visit-to-visit comparison.

Over the entire period of the study, neither atherosclerotic plaques nor carotid stenosis were observed in the control subjects. However, one of them (a 61-year-old female with hypertensive disease, smoker) suffered paresthesia persisting for several days. In another patient (a 70-year-old female with hypertensive disease, atrial fibrillation (AF) and hypercholesterolemia), both transient global amnesia (TGA) and acute myocardial infarction were diagnosed.

### ANALYSIS OF PLAQUE MORPHOLOGY CHANGES

Prospective analysis of plaque structure showed significant changes in its morphology, occurring over the whole study period. Plaques which were classified as unstable (hypoechogenic) at the beginning of our study tended to transform into stable (hyperechogenic) in later stages (except the last four months). It must be stressed, however, that all the patients in the study group were treated with high doses of statins.

Changes in the relative proportions of stable and unstable atherosclerotic plaques in the two consecutive four-month observation periods are presented in Figure 4.

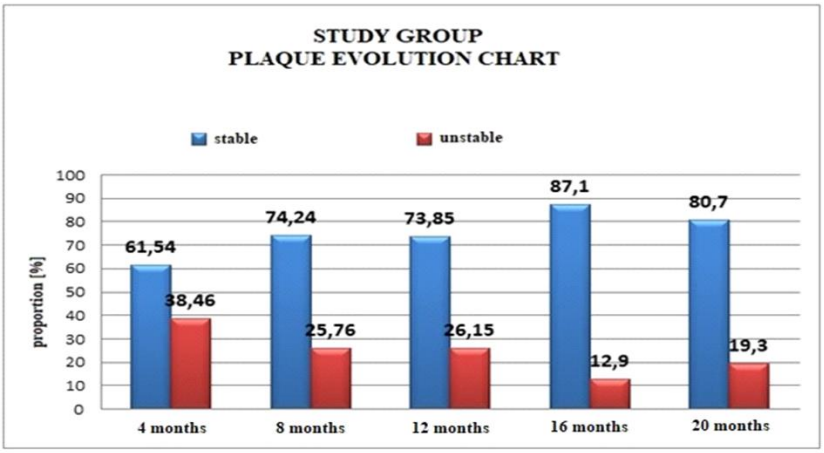


**Figure 4.** Dynamics of changes in the morphology of carotid atherosclerotic plaques over the 20-month Doppler ultrasound observation.

Statistical significance of different periods of observation varied from $p<0.001$ to $p<0.01$. The exact statistical values of changes in proportions of stable vs. unstable plaques are presented in Table 4 below.

**Table 4**. Analysis of changes in the nature of atherosclerotic plaques (stable vs. unstable) between particular visits over the 20-month observation.

| Period of study observation | Statistical evaluation |
|---|---|
| Visit 2 vs. Visit 1 | $p<0.001$ |
| Visit 3 vs. Visit 1 | $p<0.001$ |
| Visit 4 vs. Visit 1 | $p<0.001$ |
| Visit 5 vs. Visit 1 | $p<0.001$ |
| Visit 6 vs. Visit 1 | $p<0.01$ |
| Visit 3 vs. Visit 2 | $p<0.001$ |
| Visit 4 vs. Visit 2 | $p<0.001$ |
| Visit 5 vs. Visit 2 | $p<0.001$ |
| Visit 6 vs. Visit 2 | $p<0.01$ |
| Visit 4 vs. Visit 3 | $p<0.001$ |
| Visit 5 vs. Visit 3 | $p<0.001$ |
| Visit 6 vs. Visit 3 | $p<0.01$ |

EVALUATION OF IMT CHANGES OCCURRING OVER THE STUDY PERIOD

Analysis of changes in intima-media thickness did not show any statistical significance over the whole study period. The dynamics are presented in columns in Figure 5. Despite a lack of significant differences in the thickness of IMC over the 20 months, in the periods between Visit 1 and 2 (the first four months of the study) and between Visit 3 and 4 (time from the 8$^{th}$ to 12$^{th}$ month) a positive correlation (trend in the direction of changes) can be noticed, with stenosis progression (Figure 6).

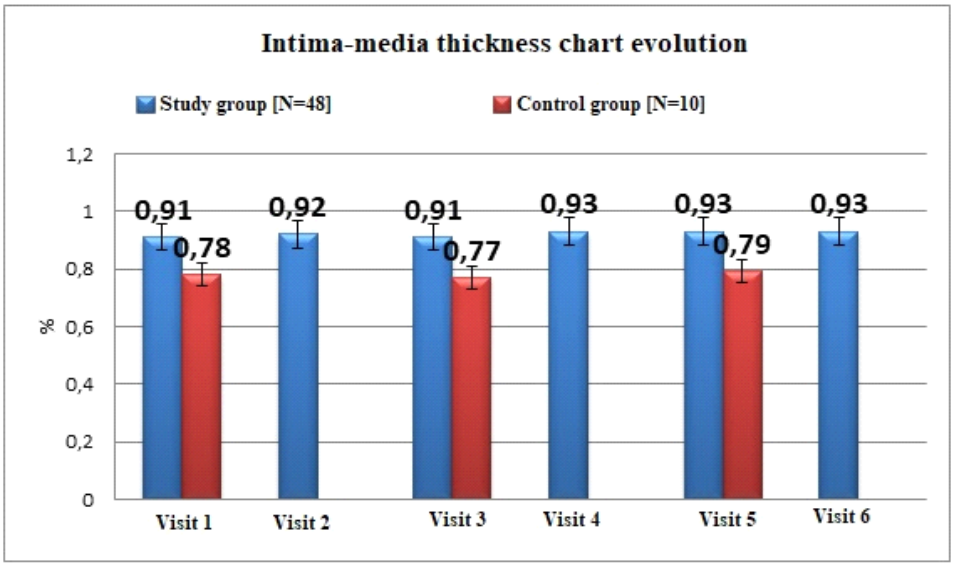


**Figure 5.** Dynamics of IMT of the carotid arteries over the 20-month Doppler ultrasound observation.

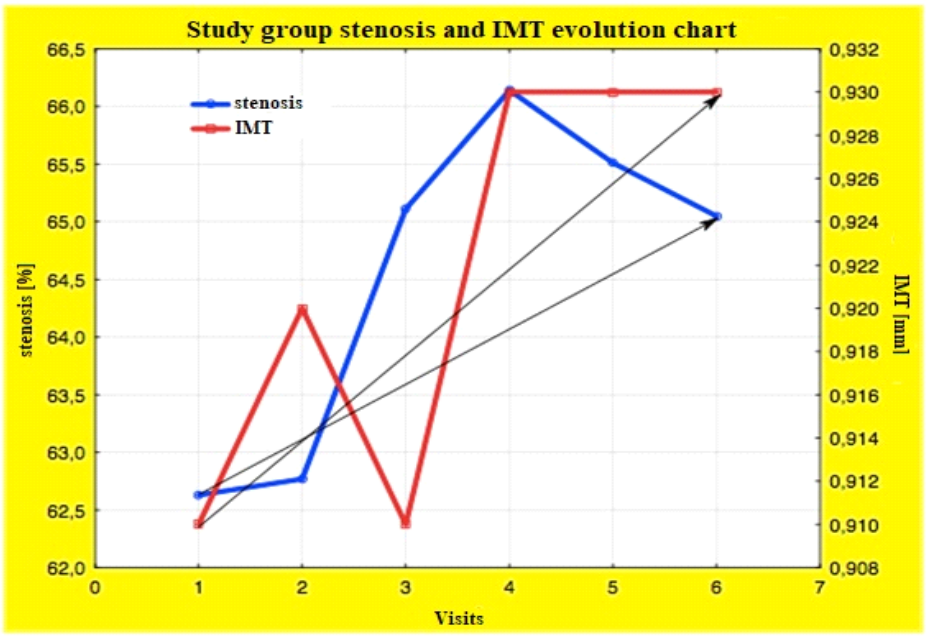


**Figure 6.** Co-visualization of the trend in the direction of IMT changes with regard to carotid stenosis changes over the 20-month period.

LABORATORY FINDINGS

Among all the laboratory tests (blood morphology, hematology, serum lipids, fasting blood glucose level, glucose loading test, HbA1c, sedimentation rate, and C-reactive protein) only changes in total cholesterol level over the 20-month observation period showed statistical significance (Table 5a, 5b). Statistically significant differences in the progression of carotid stenosis in the study group were observed between the following visits: 1 vs. 2, 1 vs. 3, 1 vs. 4, 1 vs. 5, 1 vs. 6, 2 vs. 4, and 4 vs. 5. There were no differences in cholesterol levels in the control group.

**Table 5a**. Dynamics of serum total cholesterol concentration in the study group.

| | **Study Group** | | | | | |
|---|---|---|---|---|---|---|
| **Total cholesterol (mg/dL)** | Study point **Visit 1** | Study point **Visit 2** | Study point **Visit 3** | Study point **Visit 4** | Study point **Visit 5** | Study point **Visit 6** |
| N (patients) | 48 | 48 | 48 | 47 | 46 | 41 |
| Minimum | 122.00 | 128.00 | 119.00 | 125.00 | 118.00 | 126.00 |
| Maximum | 292.00 | 274.00 | 256.00 | 293.00 | 318.00 | 278.00 |
| Median | 193.50 | 182.00 | 180.00 | 173.00 | 182.00 | 182.00 |
| Average | 198.96 | 182.44 | 177.48 | 176.26 | 187.20 | 178.02 |
| Standard deviation (SD) | 39.51 | 33.73 | 29.78 | 31.71 | 45.71 | 31.62 |
| Asymmetry index | 0.31 | 0.34 | 0.01 | 0.87 | 1.35 | 0.43 |
| Statistical evaluation | **Friedman's ANOVA test F=14.38 p<0.05**<br>**Wilcoxon Z-Test $^{1,2}$ Z = 2.76 p<0.01; Wilcoxon Z-Test $^{1,3}$ Z = 3.34 p<0.001**<br>**Wilcoxon Z-Test $^{1,4}$ Z = 3.71 p<0.001; Wilcoxon Z-Test $^{1,5}$ Z = 2.24 p<0.01**<br>**Wilcoxon Z-Test $^{1,6}$ Z = 2.46 p<0.05; Wilcoxon Z-Test $^{2,3}$ Z = 1.05 p>0.05**<br>**Wilcoxon Z-Test $^{2,4}$ Z = 2.04 p<0.05; Wilcoxon Z-Test $^{2,5}$ Z = 0.23 p>0.05**<br>**Wilcoxon Z-Test $^{2,6}$ Z = 0.58 p>0.05; Wilcoxon Z-Test $^{3,4}$ Z = 1.44 p>0.05**<br>**Wilcoxon Z-Test $^{3,5}$ Z = 1.02 p>0.05; Wilcoxon Z-Test $^{3,6}$ Z = 0.40 p>0.05**<br>**Wilcoxon Z-Test $^{4,5}$ Z = 2.36 p<0.05; Wilcoxon Z-Test $^{4,6}$ Z = 1.46 p>0.05**<br>**Wilcoxon Z-Test $^{5,6}$ Z = 0.12 p>0.05** | | | | | |

**Table 5b**. Dynamics of serum total cholesterol concentration in the control group.

| **Total cholesterol (mg/dL)** | **Control Group*** | | | | | |
|---|---|---|---|---|---|---|
| | Study point **Visit 1** | Study point **Visit 2** | Study point **Visit 3** | Study point **Visit 4** | Study point **Visit 5** | Study point **Visit 6** |
| N (patients) | 9 | 5 | 4 | 4 | 0 | 0 |
| Minimum | 143.00 | 147.00 | 140.00 | 143.00 | 0.00 | 0.00 |
| Maximum | 286.00 | 244.00 | 248.00 | 191.00 | 0.00 | 0.00 |
| Median | 170.00 | 173.00 | 162.00 | 178.50 | 0.00 | 0.00 |
| Average | 186.22 | 180.80 | 178.00 | 172.75 | 0.00 | 0.00 |
| Standard deviation (SD) | 45.22 | 37.09 | 48.06 | 21.82 | 0.00 | 0.00 |
| Asymmetry index | 1.58 | 1.70 | 1.66 | -1.11 | 0.00 | 0.00 |
| Statistical evaluation | **Friedman's ANOVA test F=1.11 p>0.05** | | | | | |

*In the control group, only the data from Visits 1-4 were analyzed.

The dynamics of changes in blood total cholesterol level over the 20-month study period are presented in Figure 7, while co-visualization of cholesterol levels and the dynamics of carotid stenosis are demonstrated in Figure 8. They showed the reverse direction of the trend of changes in these two parameters, though not statistically significant.

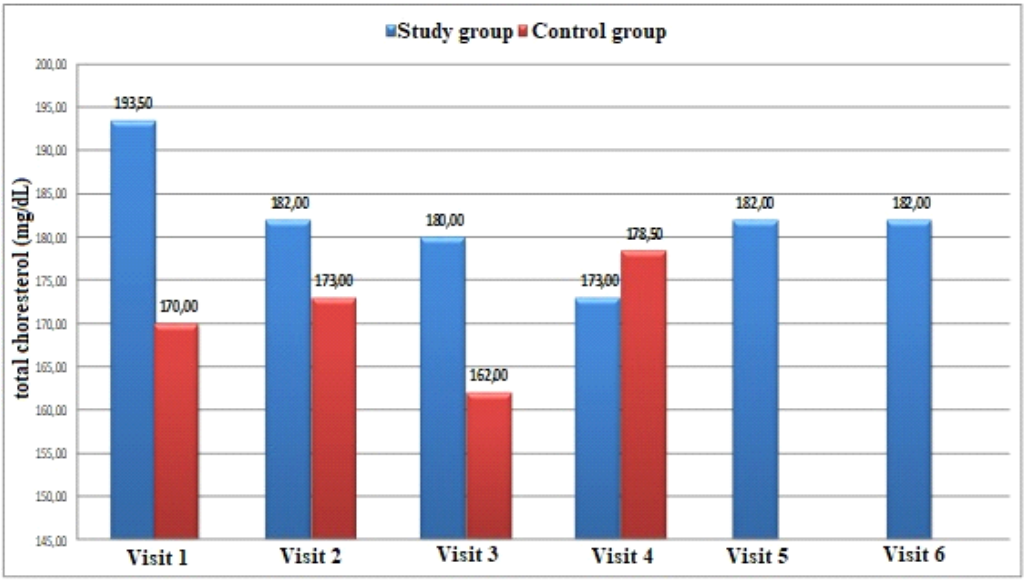


**Figure 7**. Dynamics of total cholesterol levels (on statins) over the 20-month period of observation.

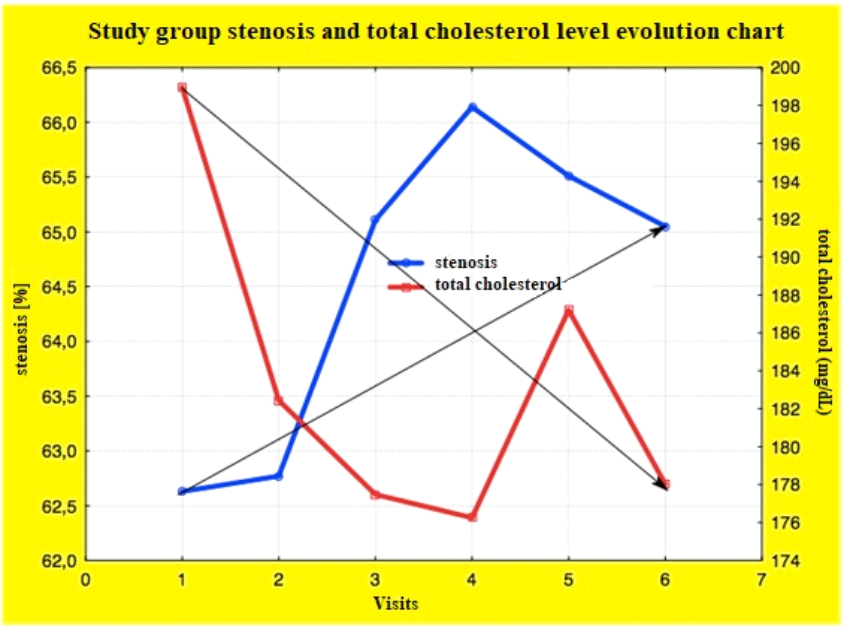


**Figure 8**. Dynamics of total cholesterol levels (on statins) over the 20-month period of observation co-visualized with changes of carotid stenosis showing the reverse direction of the trend of changes in both parameters.

We presented the same trend of dynamics by plotting the data obtained from analysis of changes in the proportion of stable vs. unstable atherosclerotic plaques in carotid arteries against concentrations of cholesterol (Figure 9). Whereas co-visualization of changes in cholesterol vs. IMT changes revealed the reverse direction in the trend of dynamics (Figure 10).

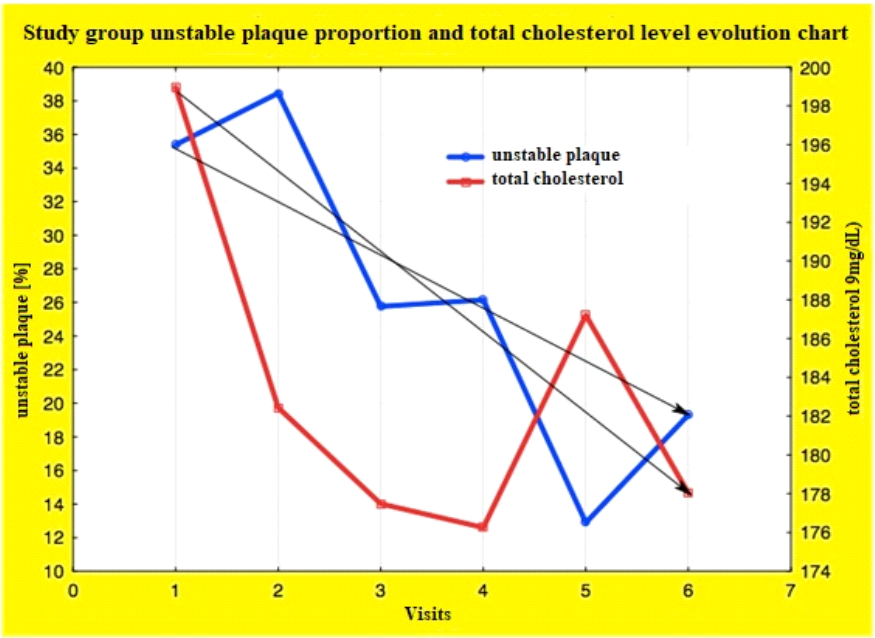


**Figure 9**. Dynamics of total cholesterol levels (on statins) over the 20-month period of observation co-visualized with changes of carotid stenosis at the same time showing the same direction of changes of both parameters.

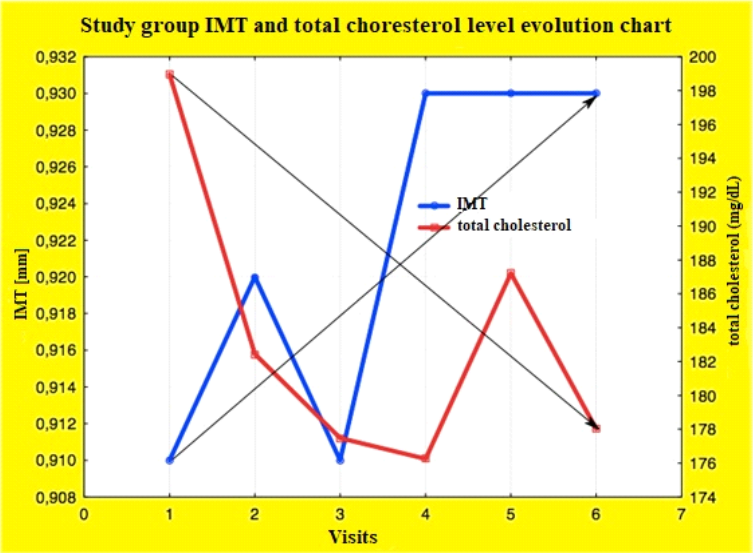


**Figure 10.** Dynamics of total cholesterol levels (on statins) over the 20-month period of observation co-visualized with changes in IMT alterations at the same time showing the reverse direction of changes in both parameters.

The results of morphology, hematology, fasting blood glucose level, glucose loading test, HbA1c, sedimentation rate and C-reactive protein did not vary between the visits within the study and control groups or between the groups.

OBSERVATIONS ON INDIVIDUAL SUBJECTS

Four participants of our study underwent endarterectomy. Those were three subjects with significant progression (over 70%) up to critical stenosis and unstable plaque structure, and one person who showed signs of critical stenosis and unstable plaque structure at the beginning of our study. No plaque progression or stroke/TIA were observed in these individuals at further stages.

Two patients developed stroke symptoms (one person with hemiparesis and aphasia, another with isolated aphasia). Both patients had unstable atherosclerotic plaques causing 50% stenosis of the carotid arteries (ICA), with no significant progression afterwards. Both patients received antiplatelet therapy (ASA).

One patient developed asymptomatic internal carotid artery occlusion (beginning from 70% stenosis).

Two patients showed "spontaneous" recanalization of a formerly occluded internal carotid artery to the degree of 90% and 95% stenosis.

## Discussion

Our study showed a significant progression of stenosis observed in atherosclerotic arteries at the initial stage. In further periods of the observation, there occurred stabilization and in some cases even a slight regression of stenosis, though not statically significant. From the methodological point of view, the best theoretical model for such as study, aimed to assess the natural course of progression of atherosclerosis and carotid stenosis, would involve comparison of two groups, i.e. stenotic patients receiving and not receiving treatment. This approach, however, would be unacceptable due to risk of serious vascular incidents with potential resultant disability or death. All our patients received the best medical treatment, namely antiplatelets combined with aggressive doses of statins, i.e. 40 mg of simvastatin or 80 mg of atorvastatin.

Most of the studies that analyze statins and their effect on stenosis focus on coronary arteries. One of meta-analyses proves that plaque volume is reduced by long-term therapy with high doses of statins (Tian et al., 2012). Such treatment results in obtaining an LDL level of 100 mg/dl or lower. Another meta-analysis based on 11 selected publications proves that treatment of coronary, carotid and aortic atherosclerosis may cause plaque regression (Okada et al., 2012). The mean observation period was 19.7 months, which is similar to our study. High doses of statins were sufficient to inhibit or even slightly reverse the stenotic process. Some investigators share the opinion that such an effect is strongly correlated with the dose of statins. However, a report on MRI examination of plaque diameters in blood vessel wall showed a similar effect, regardless of whether 80 mg or 20 mg of atorvastatin had been applied (Corti et al., 2005). In our stenotic patients, we observed a continuous process of stenosis progression despite statins used, at least up to the $16^{th}$ month, followed by a slight regression of atherosclerotic narrowing of the lumen of carotid arteries. This means that the effect of statins in not immediate but rather remote, and over the course of a relatively long-term treatment with high doses of the drugs, atherosclerosis may dominate over the therapeutic effect of such medicines.

So what should be done when stenosis progresses despite the pharmacological preventive treatment? Situation of symptomatic patients is more clear, as 70-99% stenosis requires carotid endarterectomy (CEA) or carotid angioplasty with stent (CAS) as a secondary prevention approach. This procedure may not be applied in the case of occlusion of a carotid artery. CEA is still a method of choice, and CAS is the second option, however, it has become more and more effective and safe over the last years. As for the time of such a procedure, following TIA occurrence, the sooner the better, whereas in the case of stroke, it should be performed within four to eight weeks.

Four of our study patients showed a rapid progression of stenosis. All of them underwent the CEA procedure. Neither stroke nor TIA were recorded at later stages of the observation period. There was no progression of carotid stenosis afterwards either.

Nevertheless, two of our patients, with 50% stenosis only and no absolute indication for CEA, suffered from stroke with aphasia or aphasia along with hemiparesis (which was probably non-cardiogenic) in the course of the observation period. Most likely the reason was destabilization of atherosclerotic plaque as a form of microangiopathy or macroangiopathy. A less possible though still probable cause was a rapid increase in stenosis progression.

Of special interest are findings obtained in one of our patients who developed asymptomatic occlusion over the time of observation, whereas in two other patients with initial unilateral occlusion of the carotid artery, spontaneous recanalization occurred. The latter proves that the intrinsic thrombotic and fibrinolytic mechanism may be sufficient for spontaneous recanalization.

The degree of CCA stenosis may be used not only as a predictive marker for the development of CVD, both in general terms and specifically for stroke, but also for studying and predicting vascular cognitive impairment and dementia (Weng Z).

The IMT parameter is often linked to progression of atherosclerosis as it reflects an increasing thickness of the arterial wall (Mackinnon et al., 2023). In fact, it is not the same as thickening of the wall resulting from accumulation of lipid deposits and formation of atherosclerotic plaques. Still, factors which cause an increase in IMT are similar to those regarded as cerebrovascular risk factors, of which hypertensive disease is the most important one. In our study, the changes in the IMT parameter were not statistically significant. Considering the observed progression of stenosis

of the examined arteries in our patients, such an inconsistency may seem unclear. In our opinion, as mentioned above, narrowing of the arterial lumen and thickening of the arterial wall in patients at risk of ischemic stroke are rather caused by the development of atherosclerotic plaques, which tend to destabilize the wall and brake, as well as formation of clots attached to ulcerated regions of the carotid or brain arteries. Thus, non-cardiogenic stroke is rather an effect of complex phenomena which occur within the arterial wall and the lumen, on the internal layer of the artery. Therefore, IMT itself may not be as crucial as the remaining abnormalities of the wall morphology. Moreover, it is supposed that the pace of thickening of the intima-media complex is relatively slow as compared to formation of atherosclerotic plaques, and even much slower than formation of an intraarterial clot. Thus, it was quite unlikely that the changes in IMT noted in our study could show significant differences over the 20-month observation period.

The aforementioned transformation of the arterial wall morphology in the context of atherosclerotic plaques seems to be a much more important parameter, or even a predictor of incoming stroke. Such transformation accounts particularly for destabilization of atherosclerotic plaque, i.e. an increase in the content of lipids, presence of intra-plaque hemorrhages, accumulation of thrombocytes, erythrocytes and leucocytes, which altogether form intraarterial clots. They may detach, either in part, entirely or even along with fragments of atherosclerotic plaques, becoming a potential embolic material in the brain arteries.

In our study, we presented statistically significant differences in the changes of morphology of atherosclerotic plaques. Throughout all the stages of our study, we demonstrated a long-lasting and beneficial change in the morphology of plaques, constantly transforming their morphology from unstable (hypoechogenic) to stable (hyperechogenic) structures. We link such a phenomenon to the stabilizing effect of statins used in high doses, which is in agreement with numerous publications reporting the same findings and properties of this class of drugs.

As for the effect of lowering total cholesterol produced by statins, we found that it remained constant over the first 16 months (four visits), and was followed by a slight increase in serum cholesterol level.

Additionally, when we attempted to plot cholesterol level findings against the other parameters studied, we drew the following conclusions:

- decrease in serum cholesterol level leads to an increasing trend of carotid stenosis progression; it may be interpreted rather as an epiphenomenon related to the “standard of care” treatment applied to all our symptomatic and asymptomatic patients with carotid stenosis;
- decrease in serum cholesterol level is parallel to the trend of changes in the carotid plaques, i.e. transformation from unstable to stable forms, which is interpreted as a desirable therapeutic effect of statins providing stabilization of atherosclerotic plaques.
- decrease in serum cholesterol level leads to an increasing trend of IMT progression, although the latter being insignificant; this finding suggests that cholesterol probably does not have a major impact on thickening of the complex intima-media, unlike in the case of an influence on a correlation between cholesterol level and the trend in stabilization of atherosclerotic plaques.

## Conclusions

To sum up, ultrasound examination performed every four months in both symptomatic and asymptomatic patients with at least 50 % carotid artery stenosis seems to be a reasonable pattern of monitoring the dynamics of atherosclerotic processes which lead to narrowing of the arterial lumen, deleterious transformation of the morphology of intima-media complex and morphology of carotid atherosclerotic plaques. However, 48 patients and 78 arteries examined in our study do not provide a sufficient amount of data that could support generalization of this thesis. A larger sample size including patients divided into two very homogenous groups and the multicenter study approach would definitely increase strength of such a statement. Diagnostic recommendations would be then also redefined. Various benefits of early diagnosis in high risk

patients cannot be overemphasized, considering
the very high mortality of stroke, huge costs of hospitalization of patients in acute phase of the condition and their rehabilitation, as well as permanent disability caused by a significant number of stroke cases. Additionally, our data confirmed that treating patients with statins may not only stabilize plaques of carotid arteries by changing them from unstable to stable, but also even regress stenosis, though not much and in a longer perspective, not immediately. Such effects were proven also by other investigators. These findings and conclusions, especially those concerning stabilization and regression of plaques with the use of high doses of statins, are in agreement with other similar studies and publications (Berro Rivera, 2024; Masana, 202;, Sarraju and Nissen, 2024; Yu et al., 2020).

Therefore, in our opinion, frequent Doppler USG examinations in patients with both symptomatic and asymptomatic carotid arterial stenosis have a great preventive value.

## References


Abd-Allah F., Abo-Krysha N., Baligh E. 2012, Carotid atherosclerosis: Socio-demographic issues, the hidden dimensions. *Perspectives in Medicine* 1, pp. 167–169.

Bluth E.I., Kay D., Merrit C.R., Sullivan M., Farr G., Mills N.L., Foreman M., Sloan K., Schlater M., Steward J. 1986. Sonographic Characterization of carotid plaque: detection of hemorrhage. *American Roentgen Ray Society* 146(5), pp. 1061–1065.

Corti R., Fuster V., Fayad Z.A., Worthley S.G., Helft G., Chaplin W.F., Muntwyler J., Viles-Gonzalez J.F., Weinberger J., Smith D.A., Mizsei G., Badimon J.J. 2005, Effects of Aggressive Versus Conventional Lipid-Lowering Therapy by Simvastatin on Human Atherosclerotic Lesions A Prospective, Randomized, Double-Blind Trial With High-Resolution Magnetic Resonance Imaging. *JACC: Journal of the American College of Cardiology* 46, pp. 106–112.

Garvey L., Makaroun M.S., Muluk V.S., Webster M.W., Muluk S.C. 2000, Etiologic factors in progression of carotid stenosis: A 10-year study in 905 patients. *Journal of Vascular Surgery* 31(1), pp. 31–38.

Mackinnon A.D., Jerrard-Dunne P., Sitzer M., Buehler A., von Kegler S., Markus H.S. 2023. Rates and determinants of site-specific progression of carotid artery intima-media thickness: the carotid atherosclerosis progression study. *Stroke* 10, pp. 1–15. DOI: 10.1161/01.STR.0000136720.21095.f3.

Masana L., Plana N., Andreychuk N., Ibarretxe D. 2023. Lipid lowering combination therapy: From prevention to atherosclerosis plaque treatment. *Pharmacological Research* 190, article number 106738. DOI: 10.1016/j.phrs.2023.106738.

Matos J.M., Barshes N.R., Mccoy S., Pisimisis G., Fel D., Kougias P., Lin P.H., Bechara C.F. 2014. Validating common carotid stenosis by duplex ultrasound with carotid angiogram or computed tomography scan. *Journal of Vascular Surgery* 59(2), pp. 435–439. DOI: 10.1016/j.jvs.2013.08.030.

Okada K., Ueda Y., Takayama T., Honye J., Komatsu S., Yamaguchi O., Li Y., Yajima J., Takazawa K., Nanto S., Saito S., Hirayama A., Kodama K. 2012. Influence of Achieved Low-Density Lipoprotein Cholesterol Level With Atorvastatin Therapy on Stabilization of Coronary Plaques. *Circulation Journal* 76, pp. 1197–1202.

Rivera F.B., Cha S.W., Varona M.C., Fernandez Co E.M., Magalong J.V., Aparece J.P., De Oliveira-Gomes D., Kaur G., Gulati M., 2024, Atherosclerotic coronary plaque regression from lipid-lowering therapies: A meta-analysis and meta-regression. *American Journal of Preventive Cardiology* 18, article number 100645. DOI: 10.1016/j.ajpc.2024.100645.

Rothwell P.M., Howard S.C., Spence J.D. 2003. Relationship Between Blood Pressure and Stroke Risk in Patients With Symptomatic Carotid Occlusive Disease. *Stroke* 34(11), pp. 2583–2590.

Sarraju A., Nissen S.E. 2024. Atherosclerotic plaque stabilization and regression: a review of clinical evidence. *Nature Reviews Cardiology* 21(7), pp. 487–497. DOI: 10.1038/s41569-023-00979-8.

Teramoto M., Kokubo Y., Arafa A., Kashima R., Nakao Y.M., Sheerah H.A., Kataoka H. 2024. Common Carotid Artery Stenosis Degree as a Predictor of Cardiovascular Disease in a General Population: The Suita Study. *Journal of the American Heart Association* 13(1), article number 030828. DOI: 10.1161/JAHA.123.030828.

Tian J., Gu X., Sun Y., Ban X., Xiao Y., Hu S., Yu B. 2012. Effect of statin therapy on the progression of coronary atherosclerosis. *BMC Cardiovascular Disorders* 12, article number 70.

Weng Z., Cao C., Stepicheva N.A., Chen F., Foley L.M., Cao S., Bhuiyan M.I.H., Wang Q., Wang Y., Hitchens T.K., Sun D., Cao G. 2023. A Novel Needle Mouse Model of Vascular Cognitive Impairment and Dementia. *Journal of Neuroscience* 43(44), pp. 7351–7360. DOI: 10.1523/JNEUROSCI.0282-23.2023.

Yu M., Dai X., Yu L., Lu Z., Shen C., Tao X., Zhang J. 2020. Hemodynamic Change of Coronary Atherosclerotic Plaque After Statin Treatment: A Serial Follow-Up Study by Computed Tomography-Derived Fractional Flow Reserve. Journal of the American Heart Association 9(10). DOI: 10.1161/JAHA.120.015772.

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