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ORIGINAL PAPER

JMS Journal of Medical Science

Psychiatric symptoms in patients with metastatic ALK-positive NSCLC treated with third-generation ALK-inhibitors: a brief review and case series

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ABSTRACT

People treated with anaplastic lymphoma kinase (ALK) inhibitors, especially lorlatinib, have had problems with their central nervous system (CNS). According to a real-world pharmacovigilance study, the most frequent psychiatric symptoms were mood disorders, psychotic disorders, anxiety, agitation, and irritability. Still, it is not clear how ALK tyrosine kinase inhibitors (ALK-TKIs) might cause psychiatric adverse effects. However, ALK seems involved in the endocytosis of dopamine D2 receptors in response to long-term dopamine stimulation. Here, we report three cases of patients treated with ALK inhibitors in a single Italian Centre who developed psychiatric disorders.

Introduction

3–7% of non-small cell lung cancer (NSCLC) has ALK rearrangements, which are very sensitive to ALK-TKIs [1]. Crizotinib was the first TKI approved for treating advanced *ALK*-rearranged NSCLC [2]. However, observation of acquired resistance led to the development of second-generation (ceritinib, alectinib, and brigatinib) and third-generation (lorlatinib) ALK inhibitors. These drugs produced high response rates in patients who had not received treatment before and in patients who had not responded to crizotinib [3–6]. Patients treated with ALK-TKIs, especially lorlatinib, have shown occurrences of central nervous system (CNS) disorders. These include hallucinations, cognitive symptoms, mood changes, and alterations in mental status and speech [7].

Patients treated with ALK-TKIs, especially with lorlatinib, have experienced CNS disorders, including hallucinations, cognitive symptoms, mood changes, alterations in mental status, and speech problems. Nevertheless, detailed data regarding identifying the population at risk, the onset time of cognitive symptoms, or the persistence of these symptoms after discontinuing ALK-TKIs are still lacking.

Here, we report three cases of patients treated with ALK inhibitors in a single Italian Centre who developed psychiatric disorders.

This brief review and case series aims to discuss the mechanisms through which lorlatinib can cause neurocognitive side effects and how to identify and manage them promptly in clinical practice.

The intracranial efficacy of lorlatinib

Lorlatinib is a powerful third-generation ALK inhibitor designed to overcome first- and second-generation ALK-TKIs resistance mutations, including the most common G1202R mutation. Phase I and II trials have widely demonstrated the efficacy of lorlatinib in patients with brain metastases (BM) and leptomeningeal disease who experienced progression after first- or second-generation ALK-TKIs. Specifically, the phase 1 trial included 55 patients with pre-treated NSCLC with *ALK* and *ROS1* rearrangements. Among them, 39 patients (72%) had BM, with 12 out of the 39 having received no prior local treatment to the CNS. Additionally, 24 patients (19 ALK-rearranged and 5 ROS1-rearranged) had target BM. Among patients with measurable disease, the intracranial–objective response rate (IC-ORR) was 42% for *ALK*-positive and 60% for *ROS1*-positive patients [8]. In the single-arm phase 2 trial, 228 TKI-naïve or TKI-pre-treated patients with ALK-rearranges NSCLC were enrolled. Among untreated patients, 3 out of 8 had target BM, and an IC-ORR of 66.7% was achieved. Similarly, among 133 pre-treated patients, 81 had target BM, whose IC-ORR was 63% [9]. Updated results of this study showed an IC-ORR of 66.7% in those treated with one prior second-generation ALK-TKI and 54.2% in those treated with two or more second-generation TKIs [10]. Thus, the updated analysis confirms the second-line and CNS activity of lorlatinib. The phase III CROWN trial directly compared lorlatinib with crizotinib as first-line treatment in patients with *ALK*- and *ROS1*-positive metastatic NSCLC. BM were present in 38 patients treated with lorlatinib and 40 treated with crizotinib. Among those patients with measurable BM, the IC-ORR was 82% in the lorlatinib arm and 23% in the crizotinib arm, and 71% of patients who received lorlatinib had an intracranial complete response (CR) [6]. According to the recent, updated results of the CROWN trial, with a median follow-up duration of 36.7 months, lorlatinib continued to show better overall and intracranial activity compared to crizotinib. In particular, lorlatinib did not reach a median progression-free survival (PFS), while crizotinib had a median PFS of 9.3 months. The 3-year PFS rate in the lorlatinib and crizotinib groups was 64% in the lorlatinib group and 19% in the crizotinib group. The confirmed IC-ORR in those with baseline measurable BM was 83.3% and 23.1%, respectively [11].

Lorlatinib and the blood-brain barrier

To better clarify the distribution of lorlatinib in the brain, cytological experiments were performed on different cell lines (human umbilical vein endothelial cells [HUVEC], human microvascular endothelial cells [HMEC-1], and human neuroblastoma cells [HCMEC/D3]).

Lorlatinib and crizotinib both demonstrated effects on endothelial cells, although lorlatinib inhibited the growth of HCMEC/D3 better than crizotinib. Furthermore, lorlatinib significantly downregulated the expression of SPP1, VEGF, TGF-β, and Claudin, increasing the permeability of the BBB. In particular, osteopontin expressed by the SPP1 gene is a neuroprotective glycoprotein that plays a crucial role in the maintenance of the BBB structure, leading to the destruction of tight junctions [12,13]. In non-human primates, a radioisotope of lorlatinib demonstrated significant and rapid brain distribution. To measure the intracranial concentrations of lorlatinib, 11C and 18F-isotopologues were prepared, and whole-body dosimetry assessments by positron emission tomography (PET) were performed. The 11C-labelled lorlatinib achieved the highest

concentrations in the cerebellum, frontal cortex and thalamus, intermediate levels in other cortical grey matter, and lowest values in the white matter [14]. Furthermore, in the abovementioned phase 1 trial investigating lorlatinib in pre-treated patients with *ALK*-positive NSCLC, four patients underwent lumbar puncture for cerebrospinal fluid (CSF) sampling. The mean concentration of lorlatinib in CSF corresponded to 75% of unbound plasma concentrations, demonstrating the high availability of the drug in the CNS [8].

Case summary

Case 1

In August 2019, a 46-year-old female patient with no history of psychiatric disorders was diagnosed with *ALK*-positive metastatic lung adenocarcinoma (bilateral lung metastases, mediastinal pathologic lymph nodes, single brain metastases, bone metastases). Initially, the BM localised in the left frontal lobe (≈1 cm) was surgically excised, and adjuvant radiotherapy on the surgical site was administered (30 Gy in 3 fractions). In September 2019, a first-line treatment with alectinib 600 mg/twice daily was started, achieving a partial response. In March 2020, a magnetic resonance imaging (MRI) revealed new lesions in CNS (right cerebellum and right frontal lobe, <5 mm) (**Figure 1A**), and the computed tomography (CT) scan evidenced progression in mediastinal lymph nodes. The treatment with alectinib was interrupted, and a second-line therapy with lorlatinib 100 mg/daily was started. Lorlatinib induced an extracranial partial response, reducing all mediastinal lymph nodes and an intracranial stable disease (**Figure 1B**).

After two months of treatment, the patient experienced a manic disorder with persecutory ideation, requiring hospitalisation in a psychiatric ward from 15th to 25th May 2020. Laboratory analyses were normal, and no other organic symptom causes were found. Although an analysis of the CSF was not performed, a correlation with BM was discarded due to the stability of the intracranial disease.

Lorlatinib was interrupted during hospitalisation and resumed on 26th May at 75 mg/daily dose. At the same time, an antipsychotic therapy with valproic acid 750 mg/twice daily and haloperidol decanoate 75 mg every four weeks was started. Despite the lorlatinib dose reduction, the patient was hospitalised again four days later, and a psychiatric rehabilitation program was commenced. This intervention led to a stabilisation of symptoms, and lorlatinib was continued at the same dose. In September 2020, the patient experienced

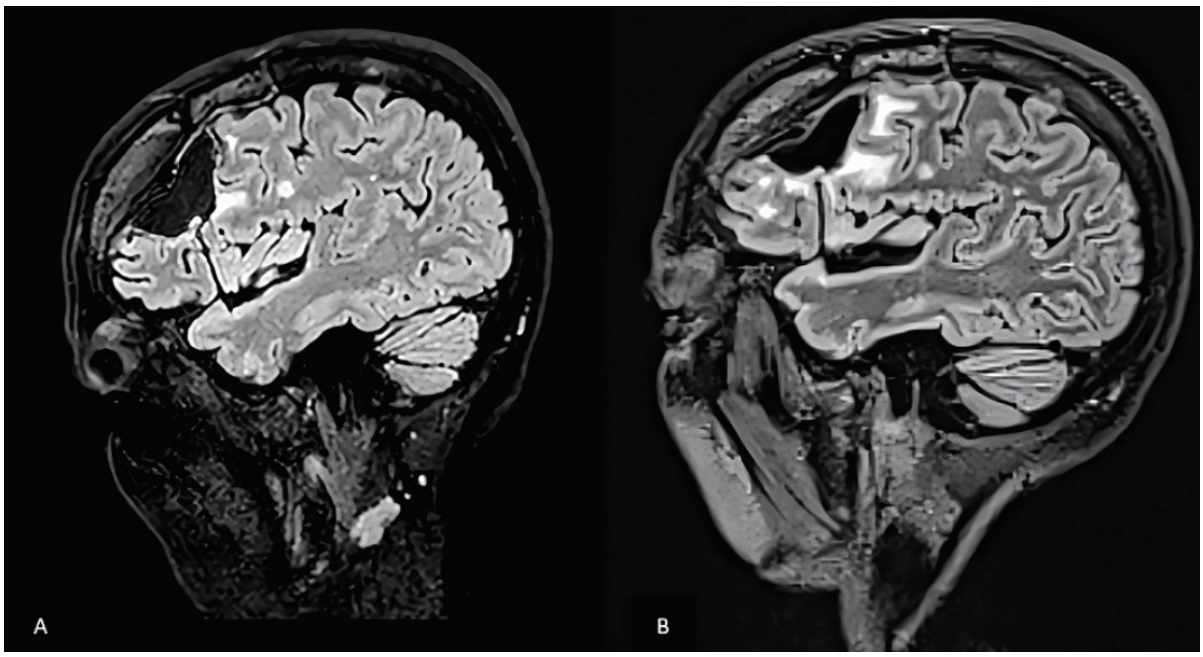

Figure 1. A: A brain MRI of case 1 was performed in March 2020, confirming a new lesion in the right frontal lobe. **B**: Brain MRI performed in May 2020 with evidence of intracranial stable disease. MRI – magnetic resonance imaging.

disease progression with the appearance of subcutaneous nodes, mediastinal lymph nodes and new BM, leading to the definitive discontinuation of lorlatinib. In December 2020, a third-line therapy with Carboplatin (AUC5) and Pemetrexed (500 mg/m^2 D1, D1=D21) was started. Unfortunately, the general conditions of the patient rapidly worsened, leading to death in February 2021.

It is noteworthy that the psychotic symptoms never resolved, and the antipsychotic therapy was never interrupted until death.

Case 2

In February 2013, a 60-year-old female patient with no history of psychiatric disorders was diagnosed with *ALK*-positive metastatic lung adenocarcinoma (bilateral lung, bone and liver metastases, two brain metastases – right frontal lobe of 1 cm, left parietal lobe of 6 mm). A first-line chemotherapy with Cisplatin (75 mg/m^2) plus Pemetrexed (500 mg/m^2 D1, D1=D21) for six cycles was administered from February to June 2013, achieving a stable disease. During the chemotherapy period, the patient underwent whole brain irradiation (WBRT; 35 Gy in 14 fractions).

In October 2013, a CT scan evidenced multisite progression, and a second-line therapy with crizotinib 250 mg/twice daily was started, achieving a stable disease as the best response. In November 2014, a single brain lesion < 1 cm in the left parietal lobe appeared, and a gamma-knife treatment was performed. In November 2015, due to a bilateral lung progression, the patient was enrolled in a phase I trial evaluating ceritinib 300 mg/daily and ribociclib 200 mg/daily, obtaining extracranial partial response and intracranial stable disease. In January 2018, an intracranial progression occurred (multiple BM < 1 cm), and a new treatment with brigatinib 180 mg/daily was started, achieving a stable disease. In October 2018, an MRI revealed diffuse brain parenchymal and leptomeningeal progression (**Figure 2: A** and **B**). Brigatinib was interrupted, and lorlatinib was started at a dose of 100 mg/daily. After 15 days of treatment with lorlatinib, the patient experienced auditory hallucinations with a suicide attempt, and she was hospitalised in a psychiatric ward from 31st December 2018 to 8th January 2019. No laboratory discrepancies were noted, and the MRI did not show evidence of progressive disease in the CNS, although an analysis of the CSF was not performed. Lorlatinib was interrupted for two months and resumed on 22nd February at a dose of 75 mg/daily. A therapy with quetiapine 25 mg

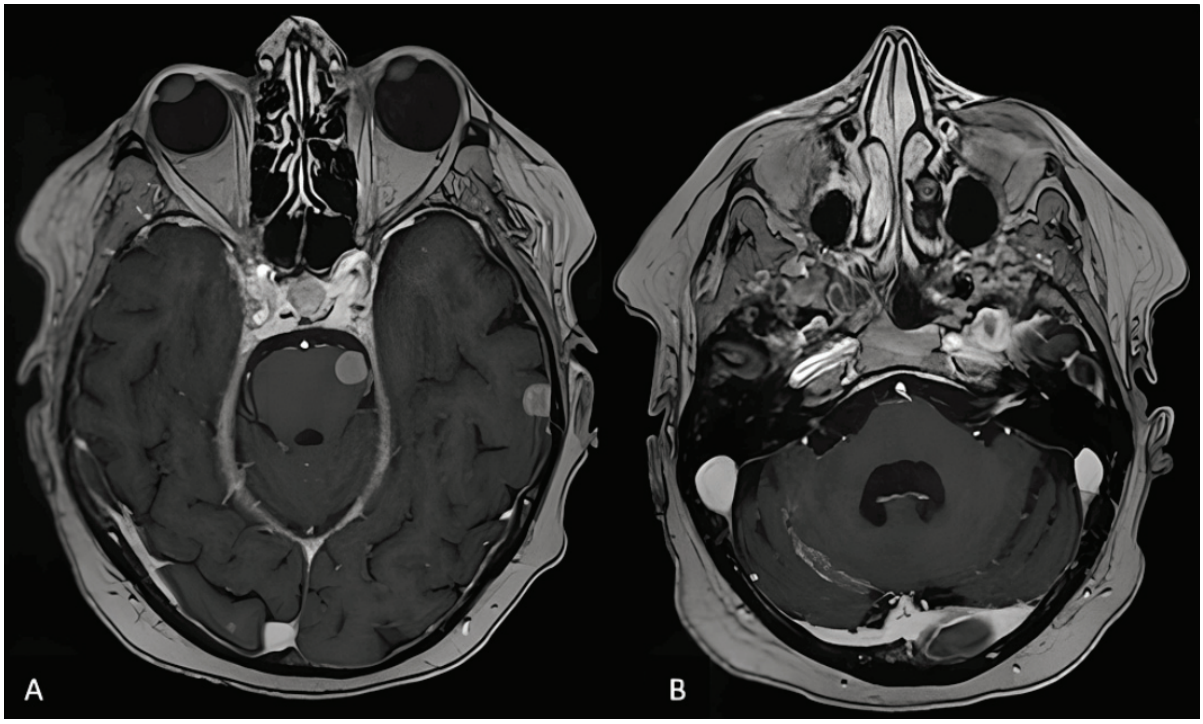

Figure 2. A: A brain MRI of case 2 was performed in October 2018 before starting lorlatinib, with evidence of pontine and left temporal brain metastases. **B**: MRI scan with evidence of cerebellar leptomeningeal carcinomatosis.

was started as well, obtaining a mild improvement of psychotic symptoms, especially auditory hallucinations. In May 2019, a new MRI showed a complete intracranial response, both parenchymal and leptomeningeal (**Figure 3: A** and **B**). Despite the lorlatinib dose reduction, the patient experienced two new psychotic episodes with visual hallucinations in May 2019 and June 2019, both requiring hospitalisation. For this reason, lorlatinib was administered at a lower dose (50 mg/daily), obtaining a stabilisation of psychotic symptoms. The antipsychotic therapy was kept unchanged and never interrupted until death, which occurred in March 2020.

Case 3

In April 2013, a 47-year-old female patient with a silent psychiatric history was diagnosed with an ALK-FISH-negative resectable NSCLC. She underwent surgery (right inferior lobectomy + lymphadenectomy; EI: pT1bN0) in June 2013. In October 2013, a pleural relapse occurred, and first-line chemotherapy with Cisplatin (80 mg/m^2 D1, D1=D21) and Gemcitabine (1250 mg/m^2 D1 and D8, D1=D21) was administered, obtaining a partial response. In February 2015, the patient experienced multisite and second-line chemotherapy with Pemetrexed (500 mg/m^2 D1, D1=D21) was administered. The benefit was maintained until October 2015, when an isolated progression on the left ovary occurred. A laparoscopic bilateral ovariectomy was performed, and the histological examination confirmed the diagnosis of *ALK*-positive lung adenocarcinoma. Two further therapies were administered: Nivolumab (240 mg flat dose D1, D1=D15) for only three months, with progressive hepatic disease as the best response, and then – from November 2016 to January 2017 – Docetaxel (75 mg/m^2 D1, D1=D21) plus Nintedanib (200 mg/twice daily on D2 to D21) for six cycles. In February 2017, a multisite progression occurred, and a Next Generation Sequencing (NGS) analysis was performed on surgical tissue from ovariectomy, confirming an EML4-ALK fusion (Variant 1).

In March 2017, a fourth-line therapy with crizotinib 250 mg/twice daily was started, achieving a partial response. In October 2017, a brain CT scan showed multiple parenchymal lesions (**Figure 4A**). New treatment with alectinib 600 mg/twice daily was initiated, obtaining a partial extracranial response and a complete intracranial response (**Figure 4B**).

In April 2018, after five months of treatment with alectinib, the patient experienced a type I bipolar disorder, necessitating hospitalisations in April 2018, October 2018, and January 2019.

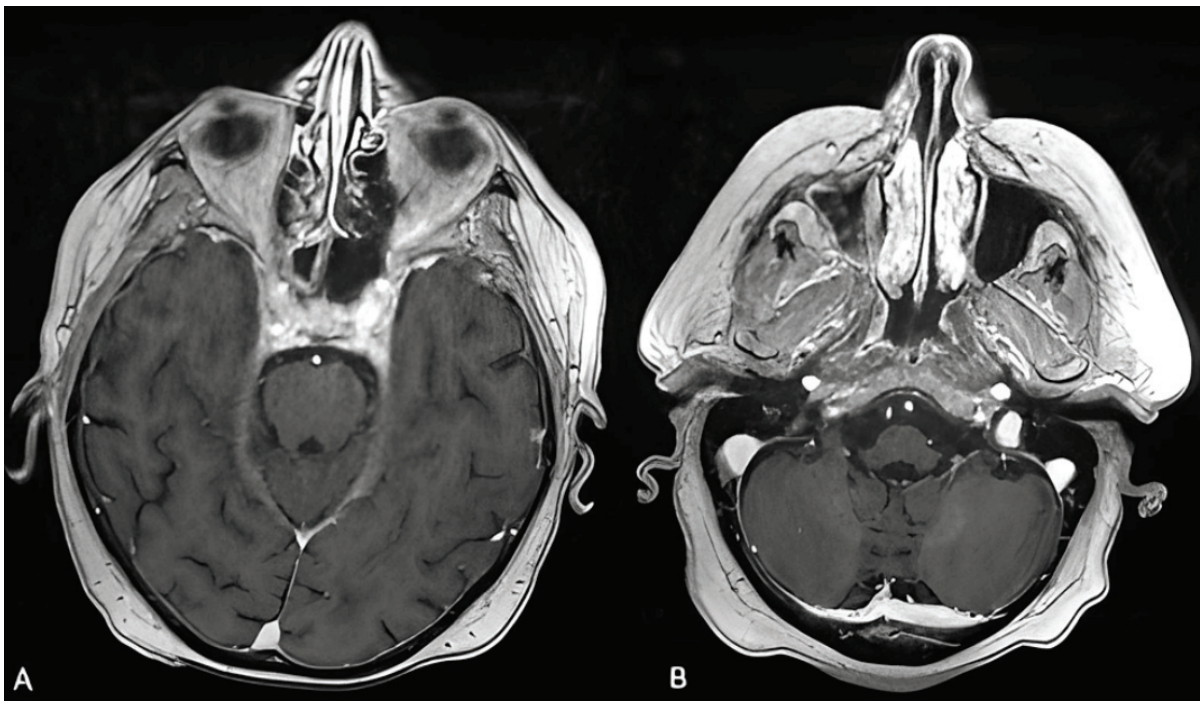

Figure 3. A: A brain MRI of case 2 with evidence of complete intracranial parenchymal response. **B**: Brain MRI scan with evidence of complete leptomeningeal response.

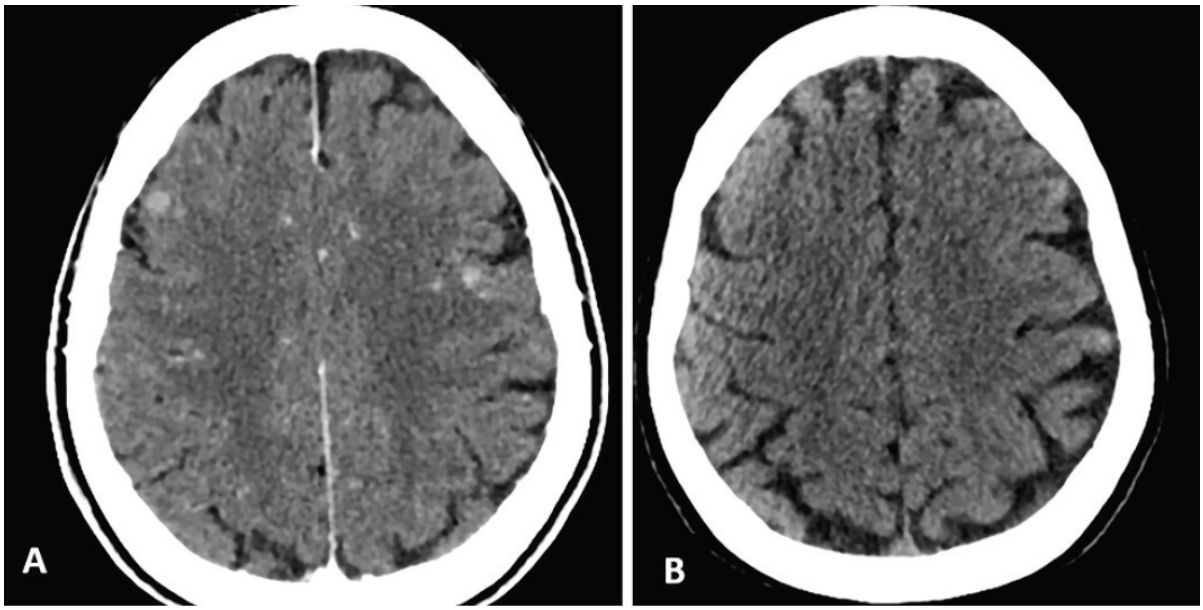

Figure 4. A: A brain CT scan of case 3 performed in October 2017 confirmed multiple parenchymal lesions. **B**: Brain CT scan performed in June 2018 with evidence of intracranial complete response. CT scan – computed tomography scan.

No laboratory discrepancies were noted, and the MRI did not indicate a progressive CNS disease. Alectinib was interrupted in February 2019, and an antipsychotic therapy with aripiprazole 30 mg and haloperidol 10 mg/ml 25 oral drops daily was set, resulting in a beneficial effect on psychiatric symptoms. In August 2019, after evidence of brain progression, a further line with brigatinib 180 mg/daily was initiated, but no other psychotic symptoms were observed. The benefit was maintained until February 2021, when a new CNS progression occurred, and therapy with lorlatinib 100 mg/daily was set. After two months of treatment, the patient experienced an exacerbation of bipolar disorder with a mild maniac episode, which required hospitalisation. Lorlatinib was reduced at a dose of 50 mg/daily with mild benefit on psychiatric symptoms. In June 2022, an isolated progression in the left parietal lobe occurred, and a surgical exeresis was performed. In February 2023, the patient experienced a new episode of bipolar disorder with manic decompensation and restless leg syndrome, requiring hospitalisation in a psychiatric ward. A different antipsychotic therapy was started, including olanzapine 7,5 mg/daily, diazepam 5 mg/ml 15 drops/daily and pramipexol 0,26 mg/daily, while lorlatinib was continued at the same dose of 50 mg daily. The patient is still alive, and the antipsychotic therapy was never interrupted.

Discussion

Lorlatinib represents a promising therapy for patients with *ALK*-positive advanced NSCLC. However, it is correlated with increased psychiatric and neurocognitive symptoms not seen as commonly with other TKIs.

As specified by pivotal trials, the most common adverse events (AEs) observed with lorlatinib were diarrhoea (up to 67%), nausea (up to 83%), vomiting (up to 67%), increased alanine and aspartate aminotransferase (up to 60%) and fatigue (up to 43%) [15].

Based on a real-world pharmacovigilance study, the most frequent psychiatric symptoms were mood disorders, psychotic disorders, anxiety, agitation, and irritability [16]. Notably, these symptoms appear to be more frequent in the female sex. According to this report, the preponderance of psychiatric reports is 2.3% for lorlatinib, 1.2% for brigatinib, 0.6% for ceritinib and 0.3% for crizotinib. A safety analysis of the phase I/II trial of lorlatinib (n = 295) reported a spectrum of CNS adverse events in 23.1% of patients, including memory impairment, confusion, and hallucinations (1.7% of grade 3–4). According to the same analysis, among the CNS disorders caused by lorlatinib, the median time to onset of mood effects was 43 days (range: 1–452 days), cognitive effects 53 days (range: 1–423) and

speech effects 42 days (range: 1–404) [17]. Furthermore, a recent case report reported the onset of paranoia and hallucinations in a 45-year-old woman with no prior psychiatric history [18].

Table 1 reports all the AEs for administering different ALK—TKIs in cases 1, 2, and 3.

was administered. It is worth noting that, in all three mentioned cases, reducing the dose of lorlatinib resulted in a mild improvement of psychotic symptoms without ever achieving resolution. Furthermore, according to the pharmacovigilance study by Sisi et al., psychotic disorders are more

Table 1. Reported adverse events after administration of first – (crizotinib), second – (ceritinib, brigatinib, and alectinib) and third-generation ALK inhibitors in cases 1, 2 and 3.

	CASE 1	CASE 2	CASE 3
Crizotinib	NA	NR	NR
Ceritinib	NA	Hypertransaminasemia G2, diarrhea G2	NA
Brigatinib	NA	Hyperlipasemia G3, hyperamylasaemia G2	NR
Alectinib	Hypertransaminasemia G2	NA	Bipolar disorder G4
Lorlatinib	Manic disorder G4	Visual hallucinations G4, auditory hallucinations G4, suicide attempt G4, hypertriglyceridemia G2, hypercholesterolemia G1	Bipolar disorder G4, restless leg syndrome G3

NA – not administered; NR – not reported.

The mechanism through which ALK-TKIs may cause psychiatric AEs remains unclear. However, there is a suggestion that ALK might be involved in dopamine D2 receptor (D2R) endocytosis in response to prolonged dopamine stimulation [19]. D2R is a G-protein-coupled receptor that regulates many CNS aspects, including cognition, mood, and reward systems [20]. This receptor is widely distributed in the brain, particularly in the striatum and nucleus accumbens. Furthermore, D2R serves as one of the primary therapeutic targets for typical and atypical antipsychotic drugs commonly employed in the treatment of neuropsychiatric disorders, including schizophrenia [21,22]. To the best of our knowledge, ALK-TKIs block the activation of ALK, increasing the expression of D2R in firing dopaminergic neurons and arousing psychotic effects [11]. According to these data, ALK-TKIs have a reverse action mechanism on D2R if compared with typical and atypical antipsychotic drugs [14]. All the cases reported here were treated with both typical (haloperidol) and atypical antipsychotics (quetiapine). This treatment regimen restored regular cognitive activity and was consistently maintained if an ALK-TKI

frequent in patients treated with lorlatinib compared to other ALK-TKIs. This is likely attributed to its enhanced ability to cross the BBB, leading to higher concentrations in the CNS [9]. Nevertheless, in patients with BM, it is challenging to attribute the onset of psychotic symptoms either to CNS involvement (brain or leptomeningeal) or to a pharmacologic class effect.

A recent study analysed safety outcomes from two large cohorts comprising more than 350 patients with ALK- and ROS1-positive NSCLC. The aim was to describe the potential association between baseline clinical characteristics (comorbidities, disease localisation and treatment, baseline medications) and the risk of developing neurocognitive adverse events (NAEs) during treatment with lorlatinib [23]. Records from patients who received lorlatinib through prospective studies at Massachusetts General Hospital (MGH, n 124) or in the phase 1/2 B7461001 (NCT01970865; n 248) study were reviewed. Most patients experienced an NAE (MGH: 60%, B7461001: 49%), although psychotic effects were infrequent (MGH: 3%, B7461001: 9%). All patients experiencing this toxicity in the MGH cohort

required dose interruption or reduction, and in all cases, the psychotic effects (specifically hallucinations) improved but did not resolve. Conversely, in the B7461001 cohort, dose interruption was needed in 27% of patients, whereas dose reduction was documented in 18%. Notably, BM ($p = 0.008$), brain radiation ($p = 0.033$), psychiatric illness ($p = 0.008$), psychiatric medications ($p = 0.001$), antiepileptics ($p = 0.001$), and stimulants ($p = 0.026$) were associated with developing cognitive effects in B7461001. These findings suggest that the presence of BM may contribute to the development of NAEs, but they are not the only factor which can justify the rise of lorlatinib-related NAEs. Particularly, a further disruption of the BBB resulting from surgery or radiation therapy may increase the risk of developing NAEs. Similarly, having a preexisting psychiatric illness is associated with a statistically significant increase in developing lorlatinib-related mood disorders in the MGH cohort [15].

Conclusions

In conclusion, in patients with BM, determining whether the onset of psychotic symptoms is correlated with CNS involvement or the use of lorlatinib is very challenging. Furthermore, a synergic effect between BM and lorlatinib cannot be excluded.

A lumbar puncture with CSF analysis should be performed to completely discard a CNS involvement, particularly a leptomeningeal spread. Unfortunately, in the three cases reported here, none of the patients underwent lumbar puncture, and attributing the onset of psychotic symptoms strictly to a pharmacological class effect remains an unresolved issue. It is worth noting that a disruption of the BBB resulting from surgery or radiation therapy may increase the risk of developing psychotic symptoms.

Possible effects on mood should be discussed with patients and caregivers before lorlatinib administration, and particular attention should be paid to patients with a history of psychiatric illness. Probably, assessing the risk of developing psychotic symptoms with a psychiatric team before initiating treatment with lorlatinib could be beneficial. This approach aims to promptly detect psychotic symptoms and initiate antipsychotic therapy at an early stage.

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ORIGINAL PAPER

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Investigation of *TXNIP*, *VDR* and *hOGG1* gene expression patterns and potential therapeutic targets in bladder cancer patients

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ABSTRACT

Backround. The aim of this study was to examine the expression levels of the *Thioredoxin interacting protein (TXNIP),* Vitamin D receptor (*VDR),* Human 8-oxoguanine DNA N-glycosylase 1 *(hOGG1)* genes in bladder cancer patients, according to clinical staging and determine the levels of potential therapeutic targets in serum samples.

Material and Methods. Tissue and serum samples of patients who underwent transurethral resection (TUR) between 2017 and 2018 were obtained. Levels of *TXNIP*, *hOGG1*, and *VDR* genes were assessed using Real time-polymerase chain reaction (RT-PCR), while levels of Thioredoxin (Trx), 8-hydroxy-2' -deoxyguanosine (8-OHdG), and 1,25-dihydroxyvitamin D (25(OH)D_3) were evaluated using the enzyme-linked immunosorbant assay (ELISA) method. Selenium levels were also measured using Optical Emission Spectroscopy (ICP-OES) in both tissue and serum samples. The protein-protein interactions and molecular and biological function

of the proteins were assessed using Search Tool for the Retrieval of Interacting Genes/Proteins. Statistical analysis was conducted using IBM SPSS Statistics version 20.0.
Results. The *TXNIP* gene showed higher expression in low-grade bladder cancer patients up to stage T1, but decreased in high-grade T1 and T2 stages. Both *VDR* and *hOGG1* gene expressions were consistently lower across all clinical subgroups. No significant differences were found in serum 25(OH)D_3, 8-OHdG, Hypoxia Inducible Factor 1 Alpha (HIF-1α), selenium (Se), and tissue Se levels.
Conclusions. *TXNIP* mRNA expression was remarkably lower in advanced stages. VDR and hOGG1 expression were low in all bladder cancer subgroups. These parameters could serve as potential targets for preventing or treating bladder cancer.

Introduction

According to Global Cancer Observatory (GLOBOCAN), bladder cancer ranks among the most common types of cancer globally, with a reported 573,278 new cases in 2020 [1]. Bladder cancer can manifest in various forms, including non-muscle-invasive bladder cancer (NMIBC) characterized by Ta/T1 stages, muscle-invasive bladder cancer (MIBC) progressing from T2 to T4 stages, or metastatic bladder cancer. Each type is characterized by distinct molecular drivers. While the overall recurrence rate for NMIBC varies between 60% and 70%, the rate of progression to a higher stage or grade, and metastasis, ranges from 20% to 30%. [2]. With the help of large-scale gene expression and sequencing studies, our comprehension of bladder cancer biology has improved, and this has led to the development of targeted treatments and immunotherapies that are more effective in clinical settings.

Selenoproteins may play a crucial role in preventing redox imbalance in bladder cancer. They are suggested to contribute to the anticarcinogenic activity through mechanisms involving oxidative stress and redox regulation, encoded by selenium as the 21st amino acid, selenocysteine [3]. In recent years, the thioredoxin system has emerged as a key player in oxidative stress regulation. Comprising thioredoxin (Trx), thioredoxin reductase (TrxR), and nicotinamide adenine dinucleotide phosphate (NADPH), this system is selenium-dependent. A deficiency in selenium (Se) can lead to decreased Trx antioxidant activity, heightened oxidative stress, and reduced apoptosis [4]. Within the thioredoxin family, thioredoxin-interacting protein (*TXNIP*) stands out as a significant player. *TXNIP* exhibits pro-oxidant properties and directly modulates Trx's antioxidant function by binding to its active site, thereby inhibiting its disulfide reductase activity and earning the title of an endogenous inhibitor of Trx [5,6].

Limited epidemiological studies have pointed to the selenium level in plasma or serum as a potential risk factor for bladder cancer. High selenium levels have been associated with a significant reduction in bladder cancer development risk compared to other cancer types (33% lower risk, 95% CI: 3% to 45%) [7]. The role of selenoproteins in bladder cancer remains poorly explored, and their significance in this context is not yet fully understood [8].

The Vitamin D receptor (VDR) is an essential DNA-binding transcription factor responsible for regulating the activity of vitamin D and its metabolic enzymes. According to a study, the *VDR* signaling pathway is essential for the effective induction of cell death by the *TXNIP- ASK1-JNK1* pathway, which was demonstrated using cell lines with VDR-knockout induced by CRISPR/Cas9 or siRNA [9]. DNA damage and oxidative DNA damage are commonly observed in various pathologies, including cancer [10,11].

Measuring *hOGG1* amplification is a useful tool for assessing the risk associated with oxidative DNA damage-repair changes [12]. Additionally, 8-hydroxy-2'-deoxyguanosine (8-OHdG) measurement serves as a direct marker of oxidative DNA damage, commonly employed for this purpose [13].

The aim of this study was to determine the expression levels of the *TXNIP, VDR, hOGG1* genes and to examine the levels of 25(OH)D_3, Trx, TXNIP, Hypoxia Inducible Factor 1 Alpha (HIF-1α), 8-OHdG, and Selenium in bladder cancer patients according to clinical staging.

Materials and methods

The compliance of this study with ethical principles and standards was approved by Kocaeli University, Clinical Research Ethics Committee (KÜ GOAKEK2017 / 1.6).

Sample collection

Patients who presented to Kocaeli University Faculty of Medicine Urology Outpatient Clinic were included in the study. Volunteers aged 18 and older, diagnosed with or suspected of bladder cancer, and individuals undergoing transurethral resection of bladder tumor (TUR-BT) surgery, were included in the study. Individuals diagnosed with other types of cancer were excluded from the study. Urethelial carcinoma tissues of 61 patients and normal tissues from 12 controls in a total of 73 voluntarily were included. Informed consent had obtained from all patients.

Collection of tissue samples

To stabilize and preserve cellular RNA, all of the tissues (cancer and normal) obtained by transurethral resection (TUR-BT) were immediately transferred to the RNA-Later solution (Life Tech., USA) and delivered to the -80°C refrigerator in the laboratory as soon as possible. Tissues were stored at -80°C until the day of the experiment. At the same time, all of the TUR-BT materials were routinely sent to the Department of Pathology for the determination of tumor grades and stages.

Collection of serum samples

Prior to the TUR-BT operation, blood samples were collected from the patients. Additionally, control serum samples were obtained from individuals without bladder cancer. After centrifugation, all serum samples were preserved at -80°C for subsequent utilization in ELISA analysis.

The demographic and pathological information of voluntaries was obtained from the patient files.

Tissue RT-PCR

Bladder cancer patient group is divided into 5 groups. TA-low grade (n = 18), TA-high grade (n = 6), T1-low grade (n = 8), T1-high grade (n = 14), and T2-high grade (n = 15). Total RNA isolation from bladder tissue samples belonging to the patients and control group included in the study was performed with the help of a commercial kit (Thermo Scientific™ GeneJET RNA purification kit, K0732). Concentrations and purities of total RNA samples isolated from the patients and control groups were determined by spectrophotometric (NanoDrop, Thermo Sci., USA) method before cDNA synthesis. At the same time, RNA integrity was checked by agarose gel electrophoresis.

cDNA synthesis was performed with a commercial kit from the isolated total RNA samples (High-Capacity cDNA RT Kit, Applied Biosystems, Catalog Number 43688. *TXNIP* [14], *VDR* [15], *hOGG1* [16], and β-actin genes were analysed using RT-PCR system (Bionem is exicyclertm 96 RT-PCR System, Turkey) shown in **Table 1**. The RT-PCR process for the samples was carried out following optimized protocols, and the mRNA levels of all genes were calculated using the (LightCycler Relative Quantitation Software Program), enabling relative quantitative analysis.

Table 1. The specific primer sequences (5'-3') used in the mRNA expression analysis of *Txnip*, *VDR*, *hOGG1*, and β-actin.

	Primer sequences
***TXNIP* (14)**	
Forward	5'- AGATCAGGTCTAAGCAGCAGAACA -3'
Reverse	5'-CCATATAGCAGGGAGGAGCTTC -3';
***VDR* (15)**	
Forward	5'- TGTAGAACATCTTTTGTATCAGGA -3'
Reverse	5'- AATGTAAGAAGCTGTAGCAAT- 3'
***hOGG1* (16)**	
Forward	5'-GGAAGGTGCTTGGGGAAT-3'
Reverse	5'-ACTGTCACTAGTCTCACCAG-3'
β-aktin	
Forward	5'- GACCACACCTTCTACAATGAG -3'
Reverse	5'- GCATACCCCTCGTAGATGGG -3'

Examination of parameters in serum samples by ELISA method

Serum samples obtained from individuals who were in the cancer patients and control groups included in the study were studied by the ELISA method using commercial kits. Trx (Cat No: E-EL-H1727), 8-OHdG (Cat No: E-EL-0028), and HIF-1α (Cat No: E-EL-H1277) levels were measured with ELABSCIENCE USA ELISA kit. TXNIP (Cat No: DZE201122136) was measured with SUNRED Shangai ELISA kit. Vitamin 25(OH)D_3 levels were measured by the chemiluminescence measurement method using the IDS-iSYS 25-Hydroxy

Vitamin D kit (Reference number: IS-2700S), using the IDS-iSYS in vitro diagnostic analyzer (V 4.03, UK).

Determination of Selenium in Tissue and Serum Samples by ICP-OES

The analysis of tissue and serum samples was conducted using ICP-OES (ICAP-6000, Inductively Coupled Plasma Atomic Emission Spectroscopy – iCAP 6000 – Thermo) at the Department of Biophysics, Istanbul University. The ICP-OES emission spectrometer equipped with a plus autosampler was computer-controlled (Thermo Fisher Scientific Inc., Istanbul, Turkey). The ICP-OES system was operated under appropriate conditions, including the selection of suitable wavelengths for each element, such as Se at 196.090 nm. The plasma operating conditions were set at a sample flow rate and elution flow rate of 1.5 L/min, a plasma gas flow rate of 15 L/min, and an argon carrier flow rate of 0.5. The peristaltic pump was set to a speed of 100 rpm [17].

Bioinformatic and Statistical Analyses

The protein-protein interactions and molecular and biological function of the proteins were assessed using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, https://string-db.org) analyse tool. The data underwent statistical analysis using IBM SPSS 20.0 software. Data normality was assessed through Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics were used, presenting numerical variables as mean ± standard deviation or median (25–75%) based on distribution. Group comparisons utilized independent sample t-tests and one-way ANOVA for normally distributed variables, and Mann-Whitney U and Kruskal-Wallis tests for non-normally distributed ones. Multiple comparisons were performed via Tukey, Dunnett, and Dunn tests. Spearman's correlation analysis and binary logistic regression were employed. Significance was set at $p < 0.05$. Statistical power was assessed using G*Power 3.1.9.2 software, showing a power of 0.83 ($\alpha = 0.05$, effect size = 0.676).

Results

The patient group consisted of 61 individuals and was further subdivided into five groups based

Table 2. Descriptive analyses of the control and cancer groups.

	Cancer group	Control group
Patient (n)	61	12
Age (Mean – year)	67.7	64.8
Female (n, %)	4 (6.56%)	2 (16.66%)
Male (n, %)	57 (93.44%)	10 (83.34%)
Tumor Number		
Single focus **(n)**	29	
Many focuses **(n)**	32	
Tumor Size		
≤ 3 cm	23	
≥ 3 cm	38	
Histological Stage		
Ta	25 (12% Female, 88% Male)	
T1	21 (9.52% Female, 90.48% Male)	
T2	15 (6.66% Female, 93.34% Male)	
Histological Grade		
Low grade	25	
High grade	36	
Information of TUR-B		
First time	24	
2 and more than 2	37	
Lenf Node		
Ta – High grade	1	
T2 – High grade	1	

on tumor type and grade: TA low-grade (n = 18), TA high-grade (n = 6), T1 low-grade (n = 8), T1 high-grade (n = 14), and T2 high-grade (n = 15). Additionally, there was a control group of 12 individuals. The mean age of control group was 64.8 (n = 12), mean age of cancer group was 67.7 (n = 61). In the control group, the rate of males was 83.34% (n = 10), while that of females was 16.66% (n = 2). In contrast, in the cancer group, the rate of males was 93.4% (n = 57), and that of females was 6.56% (n = 4). **Table 2** presents the descriptive statistical data of the tumor tissues.

RT-PCR results of tissue samples

Bladder cancer tissue samples were divided into subgroups based on the grade specified in the pathology report, and these subgroups were compared with each other. Additionally, a control group was included, resulting in a total of 6 groups. Compared to the control group, the gene expression levels were significantly higher in the Ta-low grade, Ta-high grade, and T1-low grade groups. Conversely, the expression levels in the T1-high grade and T2-low grade groups were found to be significantly lower than those in the control, Ta-low grade, Ta-high grade, and T1-low grade groups (**Figure 1**).

Moreover, patient subgroups exhibited a statistically significant decrease in *VDR* gene expression levels ($p < 0.05$) (**Figure 2**).

Similarly, a statistically significant decrease was observed in patient subgroups' *hOGG1* gene expression levels ($p < 0.05$) (**Figure 3**).

Bioinformatic analyses

The STRING analyses indicated that two biological pathways predominantly emerged when analysing the *hOGG1, VDR* and *TXNIP*. The results showed that there was a direct relationship related with DNA repair between the *hOGG1 (OGG1)* and *VDR (CYP27B1)* protein. According to the result, the DNA repair mechanism activities were decreased during the carcinogenesis. In contrast, the *TXNIP*, play role in the oxidative stress mechanism, does not directly interact with *hOGG1 and VDR* and the mechanisms related to oxidative stress increase up to the low grade T1 stage during carcinogenesis. But after then, the level of *TXNIP* sharply decreased in the high grade T1 and T2 patients (**Figure 4**).

ELISA of serum samples

Serum samples from 12 control and 61 patient groups were used to measure the levels of TXNIP,

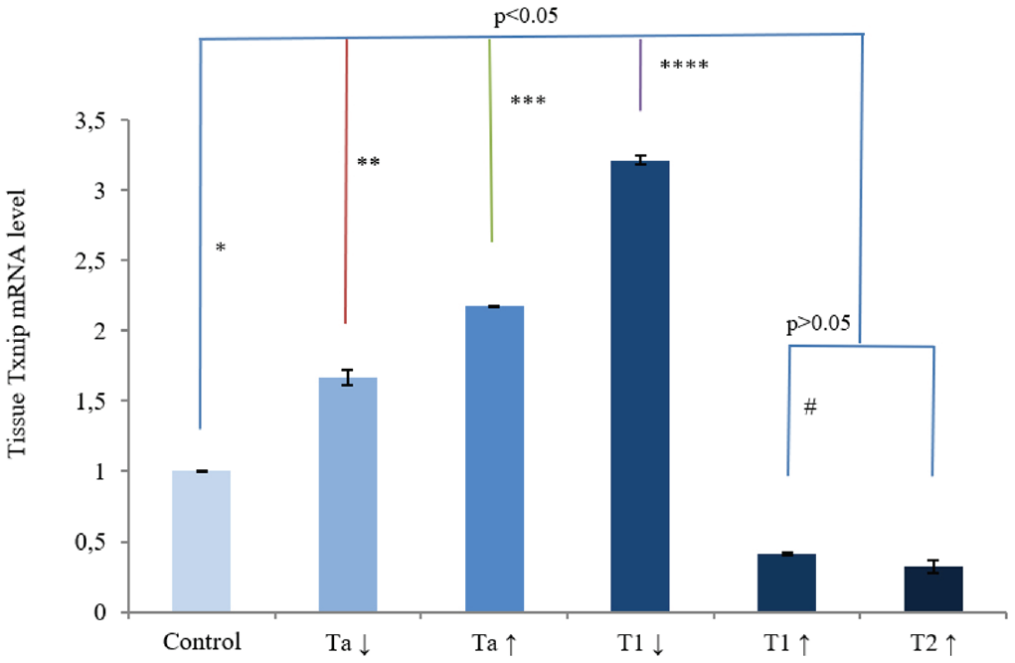

Figure 1. Tissue mRNA levels of *TXNIP gene* expressions according to tumor stage and grade. ↓, Low grade; ↑, High grade. Ta↓, Ta Low grade; Ta↑, Ta High grade; T1↓, T1 Low grade; T1↑, T1 High grade; T2↑, T2 High grade. * Control vs T1↑ and T2↑ ($p < 0.05$); ** Ta↓ vs T1↑ and T2↑ ($p < 0.05$); ***Ta↑ vs T1↑ and T2↑ ($p < 0.05$); ****T1↓ & T1↑ vs T2↑ ($p < 0.05$); # T1↑ vs T2↑ ($p > 0.05$).

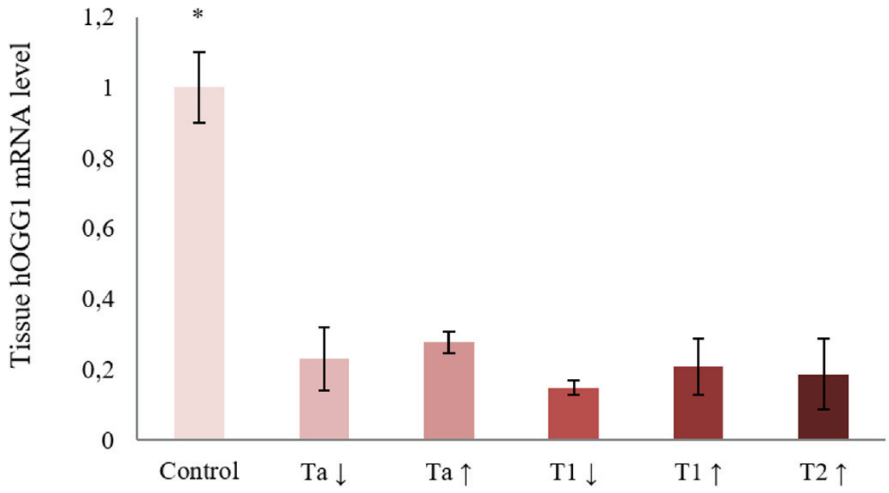

Figure 2. Tissue mRNA levels of *hOGG1 gene* expressions according to tumor stage and grade. ↓, Low grade; ↑, High grade. Ta↓, Ta Low grade; Ta↑, Ta High grade; T1↓, T1 Low grade; T1↑, T1 High grade; T2↑, T2 High grade. *Control vs Ta↓, Ta↑, T1↓, T1↑ and T2↑ ($p < 0.05$).

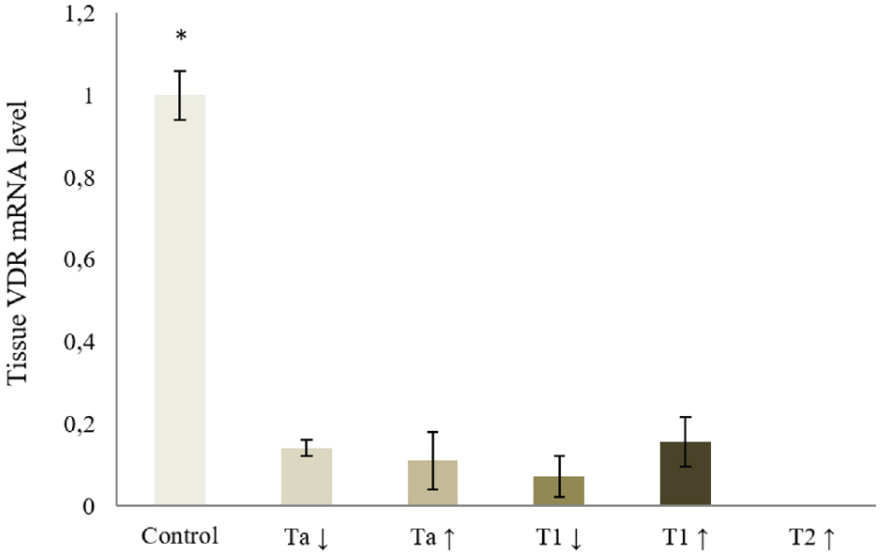

Figure 3. Tissue mRNA levels of *VDR gene* expressions according to tumor stage and grade. ↓, Low grade; ↑, High grade. Ta↓, Ta Low grade; Ta↑, Ta High grade; T1↓, T1 Low grade; T1↑, T1 High grade; T2↑, T2 High grade. *Control vs Ta↓, Ta↑, T1↓, T1↑ and T2↑ ($p < 0.05$).

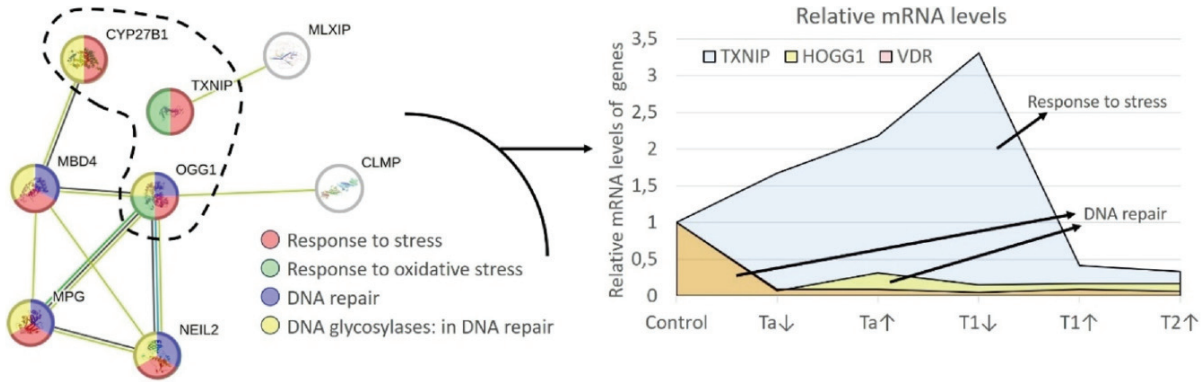

Figure 4. The illustration of the protein network revealed by STRING analysis, along with the mRNA expression of biological pathways. *VDR*; *CYP27B1*, Vitamin D Reseptor, *TXNIP*; Thioredoxin interacting protein, *OGG1*; 8-oxoguanine DNA N-glycosylase 1, MBD4; Methyl-CpG Binding Domain Protein 4, MPG; 3-Methyladenine DNA Glycosylase, NEIL2; Nei-like DNA Glycosylase 2.

Trx, 8-OHdG, HIF-1α, and 25(OH)D_3 using ELISA method (**Table 3**).

The control group had a mean age of 63.5 years (n = 12), while the patient group had a mean age of 67.2 years (n = 61). The control group consisted of 2 (16.7%) females and 10 (83.3%) males, whereas the patient group had 6 (11.11%) females and 48 (88.89%) males.

Table 3. The comparative statistical result of the serum ELISA results for the control and cancer groups.

	Control group (n = 12) Median (25–75 %)	Patient group (n = 61) Median (25–75 %)	p value
TXNIP (ng/mL)	18.45 (16.40–23.64)	14.7 (9.78–20.53)	0.100
Trx (ng/mL)	10.10 (7.45–13.55)	8.53 (3.72–13.75)	0.358
8-OHdG (ng/mL)	113.93 (103.64–120.57)	117.79 (112.45–123.29)	0.720
HIF-1α (ng/mL)	0.52 (0.33–1.16)	0.45 (0.26–0.75)	0.198
25(OH)D_3 (ng/mL)	16.6 (9.0–21.1)	14.70 (9.78–20.53)	0.861

*p < 0.05 significance level, Mann-Whitney U test was used.

Spearman's correlation test revealed that in the TA, G1 cancer group, a moderate positive correlation was observed between TXNIP and HIF-1α (r = 0.438, p = 0.036). In the TA, G3 cancer group, a strong positive correlation was found between 25(OH)D_3 and hif1a (r = 0.829, p = 0.042). In the T1-G3 cancer group, a moderate negative significance was found between HIF-1α and 25(OH)D_3 (r=-0.623, p = 0.03). No significant correlation was observed between the other parameters and subgroups (p ≥ 0.05).

Selenium level of serum and tissue

There were no differences in serum Se levels between both the control and patient groups, as well as among the different patient subgroups (p = 0.45). When tissue Se levels were compared to the control group [mean±SD: 19.042 ± 19.51, median: 11.17 (4.62–29.89 µg/ml)], no significance was found in the patient group [mean±SD: 18.07 ± 45.71, median: 3.86 (1.39–10.58) µg/ml] (p = 0.05). But there was no significant difference among the patient subgroups.

Discussion

Findings suggest that *TXNIP* mRNA gene expression is reduced in bladder cancer [18]. Research has convincingly demonstrated that the lack of *TXNIP* contributes to the progression of murine bladder cancer initiated by BBN in mice lacking *TXNIP*. Knock out TXNIP facilitates CXCR4-induced ERK phosphorylation, promoting bladder carcinogenesis. According to the same study on human bladder cancer tissues, TXNIP expression is suppressed in bladder cancers compared to normal urothelium. This suppression is particularly pronounced in high-grade and/or high-stage (pT1 or higher) cancers and could be inversely correlated to the grade and stage of bladder cancer [19]. A finding substantiated by in vitro experiments [20,21]. The decrease in gene expression in various cancer types has been documented in the literature [18,22]. Although we could not demonstrate a linear decrease between subgroups, we show that TXNIP gene expression is suppressed in the more advanced stages of bladder cancer (T1 and T2) (Figure 1). *TXNIP* levels show an increase up to T1 low-grade bladder cancer, but they significantly decrease in more advanced stages, such as T1 high-grade and T2 high-grade. Although not measured in this study, it has been reported in the literature that this induction leads to reduced Trx levels [23,24]. In recurrent bladder cancer, comprehensive gene expression profile analyses have also revealed that *TXNIP* is suppressed [25]. Bioinformatics analyses indicated that two main biological pathways emerged: stress response and DNA repair mechanisms. It has been shown that TXNIP, which plays a role in the oxidative stress mechanism, does not interact directly with hOGG1 and VDR. Additionally, this analysis revealed a direct relationship related to DNA repair between the hOGG1 (OGG1) and VDR (CYP27B1) proteins. The VDR and hOGG1 genes have been found to be directly associated with the Methyl-CpG Binding Domain Protein 4 (MBD4), 3-Methyladenine DNA Glycosylase (MPG), and Nei-like DNA Glycosylase 2 (NEIL2)

genes. According to these results, the activities of DNA repair mechanisms have decreased during the carcinogenesis process.

Based on the study findings, groups showed a deficiency in vitamin D levels. However, when examining gene expression in various cancer subgroups, the *VDR* gene expression was notably lower in the cancer groups (**Figure 1**). The T2 high-grade cancer subgroup exhibited the lowest *VDR* gene expression, almost negligible. In immunohistochemically examined bladder cancer cells, reduced VDR expression has clinically significant implications, as it may predict poorer prognosis, evidenced by lower overall survival in the study. VDR expression could serve as a potential prognostic marker in urothelial bladder cancer patients [26]. Given that vitamin D is a positive regulator of VDR expression, we cannot exclude the possibility that VDR levels in bladder cancer cells are related to and/or regulated by local and systemic vitamin D levels. Thus, lower VDR expression may reflect vitamin D deficiency, leading to poor outcomes in these bladder cancer patients [27,28].

The literature has reported that increased levels of circulating vitamin D_3 are associated with a reduced risk of various cancer types, including bladder, breast, colorectal, kidney, and prostate cancer [29]. According to the American Association of Cancer Research (AACR), vitamin D deficiency is common and has been associated with advanced stages of various cancer types. The Clinical Practice Guideline of The Endocrine Society establishes the definitions for vitamin D deficiency, insufficiency, and sufficiency, which rely on serum concentrations of 25(OH)D_3, the most commonly utilized biomarker to assess vitamin D status. According to the guideline, serum 25(OH) D_3 levels below 20 ng/mL are categorized as deficiency, levels ranging from 21 to 29 ng/mL are classified as insufficiency, and levels between 30 and 100 ng/mL are considered sufficient [30]. It has been shown that vitamin D_3 can regulate ROS levels in endometrial cancer cells by increasing TXNIP expression, resulting in the inhibition of human endometrial cancer cell proliferation [31]. In this study, serum vitamin D levels of the control and patient groups were defined as deficiency according to the guidelines. We think that the reason for the deficiency level in the control group is due to seasonal and regional factors.

One study found that the DNA repair enzyme of the Base Excision Repair (BER) pathway, *hOGG1*, which removes oxidatively damaged guanine (8-oxodG) from DNA, was reduced in leukocytes from bladder cancer patients. *OGG1* levels were examined in smokers and non-smokers [32]. It states that low OGG1 activity is associated with high levels of 8-oxoG in cancer types such as lung cancer and head and neck cancers [33–35].

On the other hand, it is noted that OGG1 activity is higher in colorectal cancer patients, which is linked to oxidative stress [36]. Additionally, another study found no significant correlation between OGG1 activity and protein levels in NMIBC tissues, suggesting that OGG1 activity can be influenced by other factors. Therefore, mRNA expression and protein levels may not reliably predict OGG1 activity [37]. It is important to emphasize that DNA repair capacity may decrease due to various factors such as smoking, obesity, etc [38]. Decreased *OGG1* expression is associated with tumor growth and progression because its ability to repair DNA can often contribute to genomic instability. In this study, decrease *hOGG1* expression was observed among all bladder cancer subgroups. We did not evaluate the risk factors in the study. Although the gene expression levels in the bladder tissue were found to be significantly low compared the control group, no difference was found in the serum levels of 8-OHdG between the control and patient group.

The level of selenium in the body has a significant impact on the growth and proliferation of cells, their mobility, development, and survival. Additionally, it is related to intercellular interactions and the redox regulation of intracellular signaling cascades involved in inflammation and apoptosis. TXNIP inactivates the anti-oxidative function of TRX by binding to the redox-active cysteine residues, causing TRX to be reversibly reduced via the actions of TRX reductase and NADPH [4]. In this context, selenium intake is necessary for the normal activity of selenoproteins, particularly GPX and TRXR, which play a role in redox regulation [39]. While the complete anticancer mechanism of Se remains unclear, it shows potential for involvement in various stages of the carcinogenic pathway [40].Although the meta-analysis of seven epidemiological studies, which included 1910 cases and 17,339 controls/

cohort members and examined the relationship between plasma or serum Se levels and bladder cancer risk, showed an inverse association between bladder cancer risk and Se levels, in this study, no significant difference was found between tissue and serum Se levels [41].

Hypoxic conditions influence factors such as angiogenesis and treatment resistance by increasing the expression of proteins, particularly HIF-1α, which are important in tumor adaptation [42,43]. In a study, it was found that the serum level of HIF-1α in the case group was significantly higher compared to the control group. When serum HIF-1α levels were compared with certain factors, such as the primary tumor, lymph node, and metastasis stages, they were found to be higher. Although serum HIF-1α was also observed to be higher in advanced urethelial bladder cancer grades, this difference was not statistically significant [44]. Similarly, in our study, no significant difference was found between the groups. When expression levels in tissue were examined, significant results were also reached in the literature [45].

Conclusion

This study highlights significant decreases in the expression of *TXNIP, VDR*, and *hOGG1* genes in bladder cancer patients, revealing their critical roles in tumor biology. Knowing the levels of Se, HIF-1α, and vitamin D contributes to the assessment of oxidative stress and cellular survival mechanisms. TXNIP is a thioredoxin-binding protein that plays an important role in regulating oxidative stress, inhibiting cell proliferation, and inducing apoptosis. Its expression is generally diminished in tumors, suggesting a tumor-suppressive function in various cancers, including bladder cancer, which has received less attention in the literature. The reduced expression of these genes is associated with the invasion of bladder cancer cells into deeper layers of the muscularis propria and the acquisition of metastatic potential. Additionally, the activity of BER proteins may serve as valuable biomarkers for prognosis, progression, and response to genotoxic therapies. Therefore, targeting *TXNIP, VDR*, and *hOGG1* is essential for improving the diagnosis and treatment of malignant tumors, particularly in bladder cancer, as it is crucial to better understand their roles and validate their potential as therapeutic targets.

Limitations

This study was conducted within a limited timeframe, which restricted the number of bladder cancer subgroups to achieve parity according to pathological results. A more detailed examination of protein levels could have been conducted.

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Conflict of interest statement
The authors declare no conflict of interest.

Author contribution
EA and MOD designed the experiments and collected data and draft manuscript preparation, visualization; EA, MS, HY, MY, FA and AME performed experiments and analysis and interpretation of Results; MOD and OD discussed critical revision or editing of the article; AME Supervised; EA, MOD, MS, HY, MY, FA, AME and OD Final approved of the version to be published.

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Ethical compliance
The compliance of this study with ethical principles and standards was approved by Kocaeli University, School of Medicine, Clinical Research Ethics Committee (KÜ GOAKEK2017 / 1.6). All participants included in this study provided their informed consent.

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ORIGINAL PAPER

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The correlation between self-efficacy, care burden, and hopelessness of mothers with medical technology-dependent children

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Keywords: technology-dependent child, mother, self-efficacy, care burden, hopelessness

ABSTRACT

Background. Having a child dependent on medical technology devices might cause some challenges for parents. Identifying these challenges and producing solutions may significantly contribute to the home care provider. This study aims to examine the correlation between self-efficacy, care burden, and hopelessness of mothers with medical technology-dependent children.

Material and methods. This study was conducted using a cross-sectional and correlational design. Between April and July 2023, 164 mothers had at least one child receiving home care and were dependent on a medical technological device. The data were collected using an information form, the general self-efficacy scale, the Burden Scale for Family Caregivers, and the Beck Hopelessness Inventory.

Results. It was found that the self-efficacy of mothers with a child dependent on a medical technological device was on the mean, their care burden was above the mean, and their hopelessness was below the mean. As the mothers' self-efficacy increased, their hopelessness and care burden decreased. It was found that the mothers' self-efficacy significantly predicted their care burden and hopelessness.

Conclusions. In the hospital setting, it is necessary to initiate training, counselling, and psychosocial support for the caregivers and the entire family. Pediatric intensive care nurses play a significant role in achieving this.

Introduction

In recent years, the survival rate of children has been rising despite prematurity, congenital diseases, asphyxia, and acute or chronic health problems [1,2]. However, this brings a new perspective on the child's health problems, and the child may require lifelong treatment/care and supervision. After undergoing prolonged treatment and care services in intensive care units, they still have ongoing requirements when they are back in the comfort of their own homes [3,4].

An atmosphere similar to home that of a hospital setting is prepared, and necessary care for the child continues at home alongside their family. These children may depend on one or more medical technological devices, either temporary or permanent. These devices include a mechanical ventilator, an oxygen condenser, hemodialysis, peritoneal dialysis, or a feeding pump [5,6]. Even though it is known that the utilisation of these devices is not particularly widespread, the number of children dependent on medical devices at home is rising each day [7]. It is estimated that 6.3–6.6 children out of every 100.000 suffer from this problem among children under the age of 16. A study conducted in the United States of America between 2009 and 2010 estimated that the rate of children dependent on medical technology and having multiple chronic diseases is approximately 11 million, with a rate of 15% [3]. An estimated 600,000 children in the United States are technology-dependent and live at home, and this population continues to grow [8].

Even though medical technological devices improve the quality of life, home care is still the leading cause of high mortality and morbidity among children [9,10]. Moreover, shifting from a hospital to home care may exceed children's and their families' standards. The families also have significant responsibilities to ensure that the treatment and care services of the child are continued [11–13]. The family needs to know how to use all the devices the child is dependent on, what to do in case of emergency response, how to respond and when to notify the emergency team. The caregivers are known to be provided with the necessary verbal and practical training before discharge from the hospital [6,14]. Given that their parents are not members of the medical team, have no medical training, and have differences in their educational, economic, and cognitive abilities, it may be challenging to meet these children's treatment and care needs in their home setting [6,10,15,16].

Parents and other healthy children might be affected in terms of the processes in the family as well as its psychological, economic, and social qualities [8,14,17]. Stress, isolation, psychosocial problems and deterioration of family relationships cause the majority of these effects. It has been reported that care burdens parents of technology-dependent children [18], leads to difficulty in adapting to care and experiences stress and depression [2]. Parents must leave their jobs to care for their children, affecting the family's economy [15,19]. Studies have reported that families feel anxious while following up on their children and have little or no sleep while monitoring, leading to their psychological disorders [8,19,20]. The study by Nishigaki et al. reported that mothers experienced social and physiological difficulties, future anxiety, and psychological disorders since caring for their children lasted for 24 hours [13]. In the literature review, the inadequacy of identifying the needs of the child and the family during the home care of children dependent on medical technological devices and the insufficiency of presenting solution proposals are remarkable.

Additionally, information was not found regarding the social support provided to children dependent on medical technology in Turkey. The necessity for a holistic, humanistic, systemic, and political approach to children who require home treatment/care and their families, the number of whom is growing worldwide, is well known. Identifying the difficulties faced by the families of medical technology-dependent children may be necessary in drawing the boundaries of these approaches. Therefore, this study examined the correlation between mothers' self-efficacy, care burden and hopelessness with medical technology-dependent children.

Material and methods

Design of the study

This cross-sectional and correlational study.

Data collection

The study was conducted with parents with at least one child receiving home care and dependent on a medical technological device between April and July 2023. Although the study population was not identified due to insufficient prevalence studies in the country, the G*Power (3.7.9.1) program was used for the sample size. The program determined the sample size was 128 individuals with an effect size of 0.3, a sampling error of 0.05, and a confidence interval of 95%. Still, the study was completed with 164 mothers, considering the missing data.

Participants and setting

It was determined that the income of the parents who could be reached was provided by the father, and the primary caregiver of the child at home was the mother. Therefore, the sample size consisted of mothers. It was conducted with mothers who have a child dependent on medical technology and live within the borders of Turkey. The inclusion criteria were determined as having a child dependent on a medical technological device, physical and mental growth retardation in the child (total dependence on the parent), and providing care for the child at home. Mothers having children who were physically, cognitively, and socially semi-dependent were excluded from the study.

Data collection tools

The Information Form, prepared by the researcher following the literature review, contains 12 questions about the socio-demographic characteristics of children and their parents [5,20].

Burden Scale for Family Caregivers (BSFC): Sarı and Başbakkal developed the scale in 2008. It is a five-point Likert-type assessment tool comprising six subscales and 43 items. Its subscales are economic burden (1, 2, 3, 4, 5, and 6), physical burden (21, 22, 23, 24, and 25), emotional burden (26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36), social burden (15, 16, 17, 18, 19, and 20), the perception of inadequacy (7, 8, 9, 10, 11, 12, 13, and 14), and time requirement (37, 38, 39, 40, 41, 42, and 43). The responses are scored by anchoring at "1 = never, 2 = rarely, 3 = sometimes, 4 = often-frequent, and 5 = always". High scores signify that the family burden is high [21]. The cut-off point is 97. The reliability coefficient of Cronbach's alpha of the scale was 93. This value was 92 in the present study.

General Self-Efficacy Scale (GSE): The original scale developed by Sherer et al. consists of 23 items. It has a structure with two factors: General Self-Efficacy (explained variance of 26.5%, Cronbach's alpha = 0.86) and Social Self-Efficacy (explained variance of 8.5%, Cronbach's alpha = 0.71). Since the items in the first factor did not point to a specific behavioural domain, the title of "General Self-Efficacy" was deemed appropriate for this factor. The factor of Social Self-efficacy reflects the expectations of competence in social circumstances. The later version of the scale, initially a 14-point scale, was converted into a five-point Likert-type scale (Sherer and Adams 1983). Yıldırım and İlhan conducted the Turkish validity and reliability study of the scale. This is a five-point Likert-type scale consisting of a total of 17 items. Each item is rated from 1 (not at all) to 5 (very good), and the total score on the scale ranges between 17 and 85 points. Items 2, 4, 5, 6, 7, 10, 11, 12, 14, 16 and 17 are reversely scored. A higher score indicates an improvement in self-efficacy belief [22]. The Cronbach's alpha reliability coefficient of the scale is reported to be 80. In this study, this value was found to be 79.

Beck Hopelessness Scale (BHS): This scale was developed by Beck et al. in 1974 to determine pessimism about the future. It consists of 20 items and is scored between 0–1. The scale applies to both adults and adolescents. The scale is scored as follows: the "no" response for items 1, 3, 5, 6, 8, 10, 13, 15 and 19 gets 1 point, and the "yes" response for items 2, 4, 7, 9, 11, 12, 14, 16, 17, 18 and 20 gets 1 point. The high score signifies that the individual has a high level of hopelessness. Cronbach's alpha reliability coefficient of the scale is 85, which was found to be 83 in this study.

Data analysis

The Shapiro-Wilk test tested the normality distribution of continuous variables. With the Shapiro-Wilk test, it was seen that the data showed a normal distribution. Pearson correlation analysis was used to investigate the relationship between numerical variables. Multiple linear regression analysis included scales with a significant Pearson correlation according to univariate analysis. In the study, the effect between the total score averages of the scale and its sub-dimensions was examined by linear regression analysis. Mean ± standard deviations (mean ± SD) were given as descriptive statistics. Statistical analysis was performed with SPSS for Windows version 25.0, and a p-value < 0.05 was accepted as statistically significant.

Ethical Considerations

The ethics committee of a university gave ethics approval for the study to be conducted on

11/11/2021 (2021/27). The necessary permissions were electronically obtained from the authors who conducted the scale's reliability and validity study. The parents signed an informed consent form before beginning the study.

Results

All of the parents who participated in the study were mothers. When the medical diagnoses of the medical technology-dependent children of these mothers were examined, it was found that 13.4% (n = 22) were diagnosed with Cerebral Palsy, 9.1% (n = 15) were not diagnosed, 8.4% (n = 14) were diagnosed with Pulmonary Failure and Spinal Muscular Atrophy (SMA), 6% (n = 10) were diagnosed with Tracheomalacia, 3.6% (n = 6) were diagnosed with muscular diseases, 3.1% (n = 5) (the same rate for each diagnosis) with Mitochondrial myopathy, Moebius Syndrome, Down Syndrome, Mucopolysaccharidosis (MPS), West Syndrome, Tay-Sachs Syndrome, and 2.4% (n = 4) (the same rate for each diagnosis) diagnosed with Nager Syndrome, Nemaline Myopathy 2, Trisomy 10q, 1.8% (n = 3) (the same rate for each diagnosis) diagnosed with Spina Bifida, Rett Syndrome, Prune 1 Syndrome, Myastenia Gravis, I-cell (Mucolipidosis Type 1), 1.2% (n = 2) (the same rate for each diagnosis) diagnosed with Walker Warburg Syndrome and Treacher Collins Syndrome, 0.6% (n = 1) (the same rate for each diagnosis) were diagnosed with Alexander Disease, Sandhoff Syndrome, MECP2 Syndrome, NCL Batten Disease, Canavan Syndrome, Ohtahara Syndrome, Lissencephaly, and Beare-Stevenson Cutis Gyrata Syndrome.

In the study, it was found that the mean age of technology-dependent children and their parents was 35.03 ± 35.43 months, the mean age of the

Table 1. Socio-demographic Characteristics of the Parents and Their Children (n = 164).

Characteristics	Mean+SD	Min–Max
Mother's age	32.82+6.11	22–58
Father's age	36.65+6.84	25–63
Child's age (month)	35.03+35.43	2–216
Number of Siblings	4.07+1.05	2–8
Child's Weight (kg)	17.76+11.57	4–65
Child's Height (cm)	94.67+23.36	47–150
Duration of diagnosis (months)	22.88+22.87	1–136
Mother's Education Level	n	%
– Literate	2	1.2
– Primary School	27	16.5
– Secondary School	80	48.8
– University	55	33.5
Father's Education Level		
– Literate	2	1.2
– Primary School	32	19.5
– Secondary School	69	42.1
– University	61	37.2
Income Status of the Family		
– Income less than their expenditures	91	55.5
– Income equal to their expenditures	58	35.4
– Income more than their expenditures	15	9.1
Child's Gender		
– Female	78	47.6
– Male	86	52.4
Medical device used by the child		
– Household ventilator	131	79.9
– Oxygen Condenser	125	76.2
– Aspirator	157	95.7
– Saturation Device	150	91.5
– Feeding Pump	120	73.2

mothers was 32.82 ± 6.11 years, the mean age of the fathers was 36.65 ± 6.84 years, and the mean number of siblings was 4.07 ± 1.05, respectively. 48.8% of the mothers and 42.1% of the fathers were secondary school graduates; 55.5% of the families had incomes less than their expenditures; 52.4% were male; 95.1% used aspirators. The mean weight of the children was 17.76 ± 11.57 kg; their mean height was 94.67 ± 23.36 cm; the duration of the diagnosis was 22.88 ± 22.87 months; and the duration of dependence on the device was 20.30 ± 21.63 months (**Table 1**).

A negative significant moderate correlation was found between the total mean score of GSE, the total mean score of BHS, and the mean score of the perception of inadequacy subscale of BSFC. A negative, weak, significant correlation was found between the total mean score of GSE, the total mean score of BSFC, and the mean scores of the economic, social, physical, emotional, and time requirement subscales (**Table 3**).

It was determined that the total score of BHS accounted for 20% of the variation in the total score of GSE, and the total score of BSFC and its subscales accounted for 9% of the variation in the total score of GSE (**Table 4**).

A one-point increase in BHS reduced GSE by -1.047 points, whereas a one-point rise in BSFC increased GSE by 0.131. One point increase in the economic burden subscale of BSFC reduced GSE by -0.117 points, one point increase in the perception of inadequacy subscale reduced GSE by -0.662 points, one point increase in the social burden subscale reduced GSE by -0.132 points, one point increase in the physical burden sub-

Table 2. Total Mean Scores of the Scales and Its Subscales.

Scales	Mean+ SD	Min–Max
TotalBHS	7.750+4.297	0.00–19.00
TotalGSE	61.609+10.055	40.00–85.00
TotalBSFC	135.829+27.028	52.00–208.
BSFC Economic burden	20.128+4.723	6.00–30.00
BSFC Perception of inadequacy	22.823+5.867	10.00–39.00
BSFC Social burden	21.737+4.116	8.00–30.00
BSFC Physical burden	13.646+4.740	7.00–25.00
BSFC Emotional burden	35.603+8.466	12.00–55.00
BSFC Time requirement	21.890+7.532	7.00–35.00

Table 3. Correlation between Total Scores of GSE-BHS-BSFC and BSFC Subscales.

	TOTAL GSE	
	r	p
Beck Hopelessness Scale (Total Score)	-.448**	.000
BSFC economic burden	-.179*	.022
BSFC perception of inadequacy	-.315**	.000
BSFC social burden	-.189*	.016
BSFC physical burden	-.227**	.004
BSFC emotional burden	-.108	.167
BSFC time requirement	-.187*	.017
BSFC Total	-.254**	.001

r – pearson correlation

Table 2 shows the mean scores on all the scales and their subscales. The total mean scores of the participants were 7.750 ± 4.297 for BHS, 61.609 ± 10.055 for GSE, and 135.829 ± 27.028 for BSFC. Their mean scores for the BSFC subscales were 20.128 ± 4.723 for economic burden, 22.823 ± 5.867 for the perception of inadequacy, 21.737 ± 4.116 for social burden, 13.646 ± 4.740 for physical burden, 35.603 ± 8.466 for emotional burden, and 21.890 ± 7.532 for time requirement, respectively (**Table 2**).

Table 4. Linear Regression.

	GSE MODEL	
	Adjusted R^2	
BHS	.195	
BSFC	.088	
	B	p
BHS	-1.047	.000
BSFC Total	0.131	.276
BSFC Economic burden	-0.117	.635
BSFC Perception of inadequacy	-0.662	.003
BSFC Social burden	-0.132	.685
BSFC Physical burden	-0.473	.047
BSFC Time requirement	-0.138	.492

scale reduced GSE by -0.473 points and one point increase in the time requirement subscale reduced GSE by -0.138 points.

Discussion

The study revealed that the duration of dependence on a medical technological device in children was 20.30+21.63 months (**Table 1**). While Düzkaya et al. reported in their research that this rate was 85.63 ± 58.4 months, the study by Berry et al. reported that 57% of the cases were younger than 12 months [23,24]. These differences are thought to be associated with the treatment and care durations of the varying diagnoses of diseases in children. However, Gökalp reported that the children were dependent on a device for an average of 25 months, similar to this study.

It was determined that the mothers with medical technology-dependent children exhibited self-efficacy above the mean (**Table 2**). Toly et al. qualitatively examined the dimension of support given to mothers with technology-dependent children. They found that mothers were supported by their spouses most beneficially, improving their self-efficacy [10]. Suzuki et al. reported in their study that both domestic support and the support of the nurse in home care brought self-confidence in mothers and child caregivers [16]. It is reported that providing economic and psychological support before the child and family shift from the hospital to home would foster caregivers [19]. When literature is reviewed, it is believed that meeting the social, economic, emotional and medical care needs of the caregivers of technology-dependent children would personally empower them and enhance the quality of care delivered to the child. This study suggests that the high self-efficacy of mothers is correlated with their attitudes towards their maternal roles and their attachment patterns to their children.

According to the literature [18,25,26], it was determined that the care burden for families with technology-dependent children was above the mean. Additionally, when the subscales of care burdens (economic burden, perception of inadequacy, social burden, physical burden, emotional burden, and time requirement) were examined, it was found that they got a score above average in all subscales (**Table 2**). In their study, Türe et al. reported that the care burden of caregivers for children with chronic diseases was mild at the rate of 38.1%, moderate at the rate of 22.9% and severe at the rate of 39% [18]. In the study conducted by Baddour et al. on the caregivers for children dependent on home ventilators due to tracheostomy, they found that many parents had to resign from their jobs or worked less to care for their children. It is known that this condition imposes an economic and psychological burden on the family [19]. Choi et al. found in their study that the caregiver cared for the child for 14 hours a day, slept for 5.6 hours, and could allocate 2.4 hours for personal care, and care burdens precluded them from meeting their individual needs [20]. In their study, Edwards et al., (found that a significant majority of families had one or more members who quit their jobs, cut down work, or took weeks of unpaid leave from their workplace to care for their children [15]. Toly et al. investigated the adaptation of families who cared for children dependent on technology at home. They found that although mothers who adapted in a short time suffered less stress, the level of their depression was high. Mothers with more extend-

ed adaptation periods suffered more stress, and the level of their depression was also high [2]. Hefner and Tsai reported that at least one of the parents was a college or a university graduate, less than half of the families had economic hardship and cared for the child for more than 16 hours, leading them to feel depressed [27]. Similar to the previous studies, this study suggests that mothers had many different care burden problems and technology-dependent children who received home care/care, and their parents could not use social support systems and health care services as desired.

It was determined that families with technology-dependent children had hopelessness below the mean (**Table 2**). Toly et al. (2019b) found that when conflicts, complications, or anything related to care developed, it was difficult for mothers to maintain a positive attitude, increasing their hopelessness [4]. Nishigaki et al. (2016) reported in their study that mothers with children dependent on medical technological devices suffered from several physiological, sociological and psychological disorders, which also caused them to feel hopeless and that they should receive professional support [13]. It is reported that mortality rates of children dependent on medical technological devices during their follow-up at home are higher than the average population, and mortality rates in the first year vary between 0–10% [9]. Gökalp (2019) reported that 16.7% of children died in four years of follow-up, Koçkar et al. (2012) reported that 12.4% of children died in the first two years of follow-up, and Hsia et al. reported that 26.6% of cases died in long-term follow-up [28–30]. Although this study did not analyse mortality rates, it can be estimated that having a child in need of permanent care may raise the fear of losing the child at any time. It is thought that these persons responsible for primary care may suffer from individual, familial, economic, social and psychological problems and require support. This can be interpreted that in case of failure to meet this support sufficiently, the feeling of hopelessness in the individual would increase, and the whole family, including the child, would be negatively affected by this.

It was also found that as the mothers' self-efficacy increased, their hopelessness and care burden decreased (**Table 3**). The regression results indicated that the self-efficacy, hopelessness and care burden of the families with children dependent on medical technological devices were significantly affected [Table 4]. Looman et al. reported a positive correlation between the quality of life of children dependent on technological devices and their parents' physical, social, and cognitive functions [31]. Hefner and Tsai reported a correlation between the financial status of the family, the depression of the caregiver, and the unmet care needs of their children [27]. It was found that as maternal self-efficacy increased, the economic burden, social burden, physical burden, emotional burden, and time requirement in the family reduced (**Table 3**). According to the regression results, it was determined that while economic burden, social burden, physical burden, and time requirement were predicted, emotional burden was not (**Table 4**). Likewise, Choi et al.. They found that more than 80% of caregivers had physical and less economic and financial burdens [20]. The study by Edwards et al. reported that the family paid 3899 US dollars (calculated as the median) for the expenditures of children dependent on a home ventilator in the last three months [15]. It was reported that the financial stress in families decreased as their income level rose, and some families suffering from financial stress reported this stress to be caused by the spending they made out of their own pockets. Mothers' self-efficacy contributes to resilience in care burden and psychosocial health. When mothers were self-efficient, they were more self-sufficient in sub-parameters related to care burden, but they were unaffected by the emotional burden. This is associated with mothers' awareness that their children depend entirely on them for their needs, and they assume a conscientious responsibility.

Limitations

The study is subjective to the mothers in the sample group and cannot be generalised to all mothers who have children dependent on medical technological devices. The limitation of the study is the exclusion of fathers from the sample group since Turkey presents a patriarchal society, and fathers tend to work. At the same time, mothers play social roles, such as being responsible for the child's care.

Conclusions

One of the most vulnerable children is technology-dependent children at home. When these children are discharged home from the hospital, the family needs to be educated about care. In the hospital setting, it is necessary to initiate training, counselling, and psychosocial support for the caregivers and the entire family. Pediatric nurses play an essential role in achieving this.

Suggestions

It is believed that mothers should be personally supported, and their self-efficacy should be further improved in their relationships/communication with their spouses and other healthy children as well as the sick child in the hospital and during home care. Finding solutions to the needs of the caregiver and the family can provide excellent and planned care so that recurrent hospitalisations of the child can be avoided.

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REVIEW PAPER

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Skin aspects of COVID-19

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ABSTRACT

Background. COVID-19 is a disease that has changed the realities of society, medical personnel, and patients worldwide. The long-term course of the COVID-19 pandemic has allowed us to observe the many changes occurring during this disease. Lesions resulting from SARS-CoV-2 infection manifest in the lungs and other systems, including the skin.

Material and methods. The paper presents a comprehensive description of skin lesions occurring. The course of COVID-19 was based on a literature review and my experience acquired in the intensive care unit.

Results. The mechanism of formation of the cutaneous manifestations in SARS-CoV-2 infected patients due to the course of the disease was discussed. Skin lesions from the treatment, hospitalisation and immobilisation of the patient were also considered. The causes of developing bedsores are also highlighted, with examples provided with photographs. In addition, the paper includes tables comparing the appearance of skin lesions with the severity of the course and caused by pharmacotherapy, which may be a practical instrument in clinical practice.

Conclusions. Cutaneous manifestations may be the single symptom of COVID-19, which may facilitate the diagnosis of this disease. It seems necessary to extend the diagnostics of skin lesions during COVID-19 to understand their pathogenesis. Inadequate care due to staff shortages or inadequate education may lead to the development of pressure sores. Implementing solutions that could protect patients and staff is imperative. In the event of future pandemics.

Introduction

COVID-19 is a disease that has changed the realities of society, medical personnel, and patients worldwide. The long-term course of the COVID-19 pandemic has allowed us to observe the many changes occurring during this disease. Patients with COVID-19 require specialised care from physicians of many specialities. Lesions resulting from SARS-CoV-2 infection manifest in the lungs and other systems, including the skin.

Vascular damage and hypercoagulability are characteristic manifestations [1] of COVID-19 disease. These results from the pathomechanisms discussed in detail in this article can cause vascular skin lesions. The clinical manifestation of skin lesions is also presented, and the different types of skin lesions are summarised along with the duration and severity of COVID-19.

This paper discusses the mechanisms of cutaneous manifestation of COVID-19, which results from hypercoagulability and endothelial dysfunction, prolonged patient stay and inadequate decubitus prophylaxis due to staff shortages, among other factors.

COVID-19 skin lesions may manifest in association with the patient's treatment.

The skin lesions observed in patients with COVID-19 were selected based on a literature review.

Material and methods

A literature search was conducted, using electronic databases PubMed and The Lancet for the terms 'COVID-19' and 'SARS-CoV-2' in combination with 'skin manifestations', 'skin lesions', 'pharmacotherapy', 'cutaneous manifestations', 'pressure ulcers', 'pressure injuries', 'endotheliopathy'. The paper was based on 80 literature sources.

Endotheliopathy and coagulopathy in the course of COVID-19

Endotheliopathy and coagulopathy are becoming increasingly common in the course of COVID-19 disease [2]. For this reason, many scientific works focus on understanding these disorders' pathophysiology. This chapter discusses the main mechanisms of derangement of hemostasis during SARS-CoV-2 infection.

SARS-CoV-2 virus penetrates human cells through the angiotensin two converting enzyme (ACE2) receptor [3]. Consequently, ACE2 receptor activity is lost, causing a reduced ability to inactivate angiotensin two and reduced production of angiotensin 1–7 [4]. The angiotensin 2 vasoconstricts severely and causes vascular inflammation. The imbalance between angiotensin 1–7 deficiency and angiotensin two hyperactivity has caused thrombosis and inflammation in experimental models [4]. A specific marker of glycocalyx degradation is angiopoietin-2. Angiopoietin-2, a crucial mediator of glycocalyx damage, regulates endothelial homeostasis, including angiogenesis and inflammation [5]. Therefore, the study conducted by Smadja et al. presents the predictive significance of angiopoietin-2 in COVID-19 patients admitted to the intensive care unit. It was shown that angiopoietin-2 level above 5000 pg/ml is a potential criterion for admission of patients to the intensive care unit (Sensitivity = 80.1%, Specificity = 70%, Positive Predictive Value = 72.7%, Negative Predictive Value = 77%) [5]. So far, it has not been shown whether endothelial damage is caused directly by SARS-CoV-2 virus infection or by congenital, autoimmune processes based on the "cytokine storm" [6]. "Cytokine storm" during COVID-19 is characterised by increased expression of IL-6 and TNF-α. Most probably, many factors influence the degree of endothelial damage and, thus, the severity of symptoms during COVID-19.

Coagulopathy associated with COVID-19 (CAC) is most commonly manifested by elevated D-dimers, products of plasmin-mediated fibrin degradation, related to the severity of COVID-19 infection. They are an independent risk factor of mortality [7]. The analysis of 1099 patients with laboratory-confirmed COVID-19 infection showed that D-dimer ≥ 0.5 mg/dL was present in 46.4% and 60% of those with severe disease status [8]. In the analysis, including 191 patients, we observed that the odds ratio (OR) of mortality for D-dimer > 0.5 µg/ml was 2.14 (p = 0.52), and for D-dimer > 1 µg/ml was 18.42 (p = 0.0033). The ACTIV-4B clinical trial showed that about 10% of all patients infected with SARS-CoV-2 have elevated D-dimer levels, demonstrating the significant role of coagulopathy in COVID-19 [9]. Fibrinogen is a glycoprotein involved in blood clotting, of which an elevated blood concentration indicates coagulopathy [10]. A study conducted by Ranucci et al. showed that at the time of admission of COVID-19 patients, fibrinogen levels were four times higher than the upper limit of normal (ULN) for fibrinogen (ULN for fibrinogen-400 mg/dL; p = 0.001) [10]. Mean activated partial thromboplastin time (APTT) was slightly prolonged in severe COVID-19 patients compared to mild (29.7 seconds vs 26.9 seconds; p = 0.0003) [11]. Thrombocytopenia (<150 × 10^9/l thrombocytes in the blood) in sepsis correlates with severity and mortality, but no such correlation was demonstrated with COVID-19 [12]. The study presented by McConnell et al. has confirmed the significant impact of interleukin-6 (IL-6) in the process of coagulopathy and endotheliopathy [13]. The study by Ranucci et al. has shown a correlation between the presence of inflammation and coagulopathy by noting high fibrinogen and high IL-6 levels in patients with confirmed SARS-CoV-2 infection [10]. In addition, IL-6 trans-signalling leads to increased production of pro-coagulation factors such as factor VIII and vWF [13].

In summary, coagulopathy and endotheliopathy occur most frequently in critically ill, hospitalised patients with COVID-19. The markers used to assess endotheliopathy are angiopoietin-2, IL-6, factor VIII, vWF, and soluble thrombomodulin [14–17]. Soluble thrombomodulin, vWF, and angiopoietin-2 are predictive markers of severe COVID-19 infection, while soluble thrombomodulin, based on vWF, is a predictive factor of mortality in patients with COVID-19 [14,18]. On the other hand, we assess coagulopathy by D-dimer level, IL-6, fibrinogen, PT and APTT time, and platelet count [8,11,19]. Only D-dimer level is a predictive factor of mortality during COVID-19 [7]. The other markers need further studies to determine their usefulness in assessing the severity of COVID-19 infection.

Cutaneous manifestations of COVID-19

The incidence of skin lesions in COVID-19 ranges from 0.2% to up to 29% in patients infected by SARS-CoV-2 [20–23]. Vesicular eruptions, maculopapular exanthema, urticarial eruptions, livedo or necrosis, and chilblain lesions are the most common skin presentations during COVID-19 [23].

According to the pathomechanism, cutaneous manifestations due to the COVID-19 course may be divided into vascular-related and inflammation-related lesions [24].

Vesicular eruptions are lesions similar to those occurring in the course of chickenpox [21]. These lesions are most commonly located on the trunk and appear as monomorphic vesicles in contrast to the polymorphic vesicles seen in chickenpox [23]. These lesions occur in approximately 15% of patients before presenting other symptoms associated with COVID-19 [23]. Vesicular eruptions resolve in approximately 10–12 days and are associated with moderate to severe SARS-CoV-2 infection [25]. The lesions may be accompanied by pruritus [26].

Maculopapular exanthema occurs along with other COVID-19 symptoms, usually in patients with severe disease. The mortality rate for patients with this type of lesion is estimated to be 2% [27]. These lesions typically appear more than 20 days after the first symptoms of COVID-19, resolve in 7–11 days, and are accompanied by pruritus in 50% of cases [23,24]. This is the most common form of skin lesions during the COVID-19 [28]. These lesions may result from a direct reaction to a viral infection or an adverse drug reaction [24,25].

Urticarial eruptions are most common on the trunk [25]. They occur in moderate to severe cases of COVID-19 and resolve after about 6–8 days,

often parallel with systematic symptoms [23,24]. Vesicular, maculopapular and urticarial eruptions are classified as lesions caused by inflammation. Due to elevated amounts of inflammatory cytokines caused by SARS-CoV-2 infection, perivascular infiltration of inflammatory cells develops—consequently, a dilatation of vascular vessels and oedema result in inducing skin eruptions formation. ACE2-related mechanisms and the direct effect of the SARS-CoV-2 virus on the epidermis (basal layer and keratinocytes) also have additional influence, which is also responsible for this type of skin manifestation.

Livedo or necrosis and other symptoms of COVID-19 [23]. These lesions are secondary to COVID-19-induced thrombotic vasculopathy [25] occur. They have been observed to occur most frequently in elderly patients who undergo severe disease. They are characterised by a high mortality rate of approximately 10% [26]. Chilblain (COVID Toes) are macular lesions resembling frostbite, occurring in young patients with a mild to asymptomatic disease course (24,29,30). Skin symptoms resolve in about 12–14 days, and 1/3 of people with these lesions experience pain and itching [31].

The severity of infection is related to the type of skin lesions observed [32]. From the mildest course of COVID-19 in people with chilblain to those requiring hospitalisation and intensive care with livedo or necrosis lesions [23].

Skin lesions can be divided into early and late onset, depending on their onset. Early manifestations include urticaria eruptions and maculopapular exanthema, whereas late lesions include chilblain [33].

Depending on the time of appearance of the skin lesions, the nature of the lesions can be determined. Lesions that appear up to 7 days after the onset of the first symptoms of COVID-19 usually have the character of a viral rash (**Figure 1**), whereas lesions that appear seven days after the first symptoms of SARS-CoV-2 infection typically have a vascular origin [34]. Lesions of vascular origin include livedo or necrosis and chilblain lesions [34]. A summary of skin lesions during COVID-19 is shown in **Table 1**.

Among the lesions observed during COVID-19, noteworthy are those formed due to the so-called "skin failure." These lesions develop during the acute, critical period of hypoperfusion and multi-organ failure. They often assume a butter-

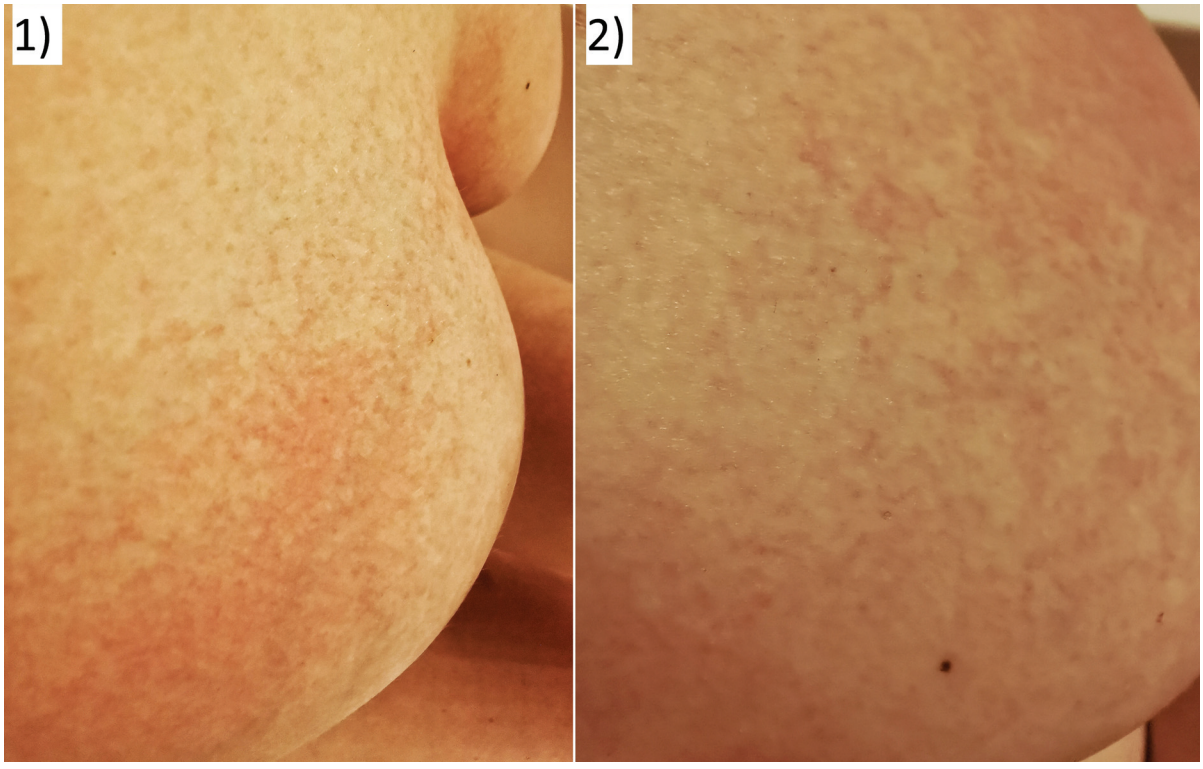

Figure 1. Viral exanthem during COVID-19 localised on the breasts.

Table 1. Summary of skin lesions in the course of COVID-19.

Cutaneous manifestation	Potential causes in COVID-19	Time to lesions resolve	Frequency	Course of COVID-19	Most common localization	Associated cutaneous symptoms	Association with other COVID-19 symptoms	Mortality	Differential diagnosis	References
Inflammatory and exanthematous rashes										
Vesicular eruptions (+ papulovesicular exanthem/varicella-like lesions)	direct viral damage to basal keratinocytes	8–12 days	9%	moderate to severe	trunk	pruritus	3 days after systemic symptoms	lack of data	Herpes infections and Grover's disease[2]	24–27
Maculopapular exanthema	drug-induced or a direct reaction to a viral infection	7–11 days	47%	severe	trunk	pruritus	more than 20 days after the first symptoms of COVID-19; simultaneously or immediately after other symptoms of the disease	2%	other viral rashes and drug-induced skin reactions	23, 24, 27, 36
Urticarial eruptions/rash	drug-induced or "cytokine storm"; nonspecific mast cell activation, direct endothelial damage, antigen-antibody deposits, activation of complement, activation of the kinin pathway	6–8 days	16–19%	moderate to severe	trunk and extremities	pruritus	onset concurrently with systemic symptoms	2%	drug-induced skin reactions	34, 38, 39, 50
Vasculopathic lesions										
A diverse group: asymptomatic patients, sparse patients, intensive care unit patients										
Lesions may result from: coagulation disorders and consequently microemboli, which may lead to disseminated intravascular coagulation (DIC) in severe cases; vasculitis due to small vessel occlusion, a neurogenic, microthrombotic mechanism mediated by immune complexes										
Livedo (livedo reticularis + livedo racemosa) or necrosis	secondary to COVID-19-induced thrombotic vasculopathy	variable	6%	more severe	extremities	pain, burning, itching	at any time during SARS-CoV-2 infection differential symptoms	10%	antiphospholipid syndrome, lupus erythematosus cutaneous panniculitis, cryofibrinogenemia,	23, 26, 27, 80–83
Ischemic acral lesions: chilblain-like + acral ulcers	without cold exposure or other predisposing substrates	2–8 weeks	pseudo-chilblain – 19%; 19–40% of adults with a milder course, in 16% of those hospitalized	chilblain mild to asymptomatic course; an acral ulcer occurs in critically ill patients	toes	pain, itching, cold sensation	on average after 9 days, frequently late in the after other symptoms, even after a recovery period	lack of data	Cold injuries, systemic lupus erythematosus	23, 24, 27, 36, 84, 85

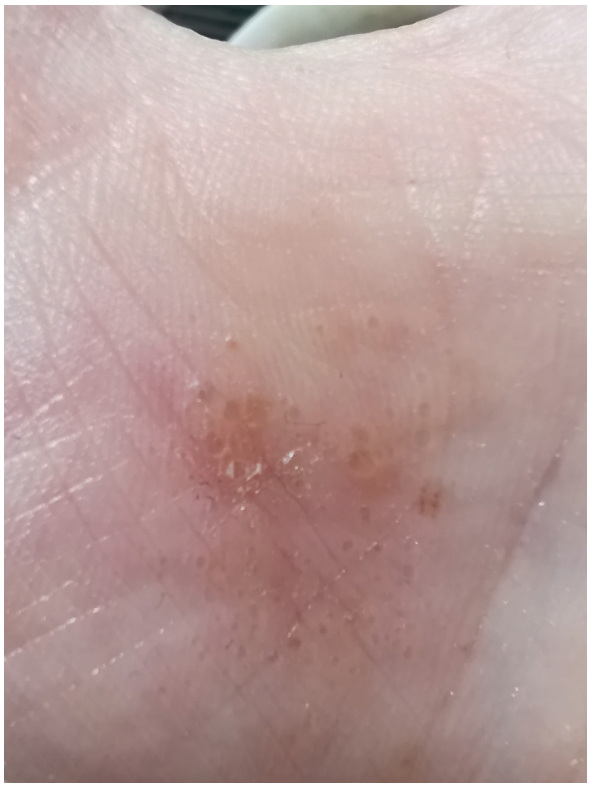

Figure 2. Contact dermatitis located on the palm.

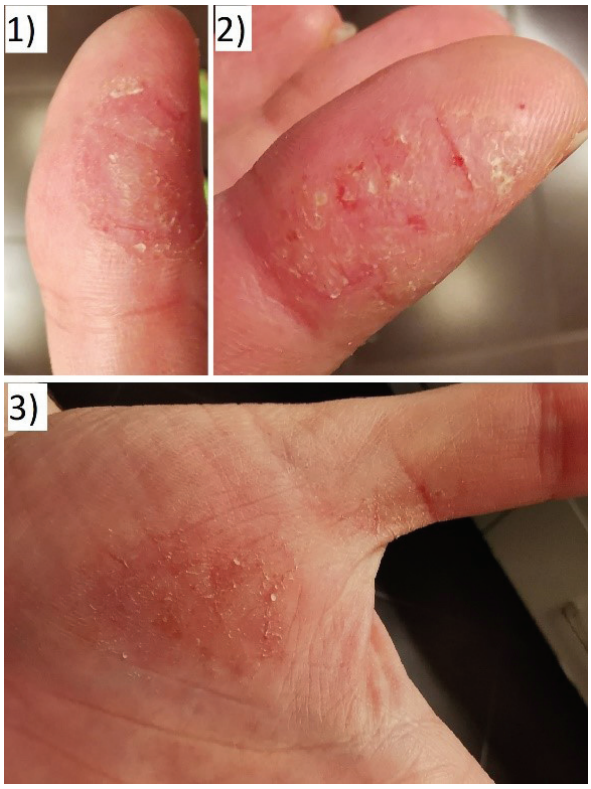

Figure 3. Atopic dermatitis flare - hand eczema.

fly or pear shape, rapidly progressing from hyperpigmentation to necrosis [35].

Other cutaneous manifestations were also observed that may be due to factors such as disinfectant-induced contact dermatitis/emphysema (**Figure 2**), telegonous alopecia, alopecia areata, nail changes, Raynaud's phenomenon-like lesions, and bedsores in hospitalised patients [27,36].

An important aspect to raise regarding skin lesions is atopic dermatitis (AD). During the COVID-19 pandemic, an increase in the frequency of exacerbations of atopic dermatitis with mild clinical severity was observed [37]. **Figures 2** and **3** show an example of cutaneous manifestations of SARS-CoV-2 infection in a patient with atopic dermatitis. The course and severity of AD are determined by many factors, including genetic, environmental, and immunological. AD patients develop type I immune hyperreactivity and increased production of Th2-type cytokines (such as IL-4, IL-5 and IL-6). SARS-CoV-2 infection can also result in an increased immune response, leading to the overproduction of inflammatory mediators (specifically TNF-α, IL-1, IL-8, and IL-6, as in AD). In COVID-19, as in AD, a comparable immune effect is observed in the form of increased overproduction of cytokines, which may lead the organism to perceive SARS-CoV-2 infection as another exacerbation of AD [38].

Skin lesions caused by treatment

The differential diagnosis of skin lesions should also consider adverse skin reactions caused by pharmacotherapy during COVID-19. Selected medications are discussed below.

Systemic glucocorticosteroids commonly used in hospital treatment could cause adverse cutaneous events such as pruritus, burning, erythema, oedema, fissures, urticaria, papulopustular lesions, telangiectasia or purpura [39,40].

Most guidelines recommend using anticoagulants, and according to some studies, adjunctive therapy with low molecular weight heparin (LMWH) may be associated with lower patient mortality [41]. However, LMWH use may be related to heparin-induced skin necrosis at the injection site or a distance, manifesting as erythematous plaques, necrotic ulcers, hemorrhagic blisters, and petechiae. Cases of fixed erythema after

enoxaparin administration have also been reported [42,43]. Side effects of antiretrovirals, such as ritonavir or lopinavir, may manifest as maculopapular rash, exfoliative erythroderma, Stevens-Johnson syndrome or toxic epidermal necrolysis, or scleroderma-like lesions, annular erythema and pruritus, urticaria or drug eruptions [39,40]. A symptom related to remdensivir may be a maculopapular rash [40,42,43]. Rush, including urticaria and pruritus, are the cutaneous side effects of Sotrovimab [44].

Tocilizumab is a humanised monoclonal antibody against IL-6 receptors and can potentially cause pruritus, *psoriasiform dermatitis*, maculopapular rash, urticaria and pustular eruptions [39,40]. Anakinra, on the other hand, may cause a generalised urticarial rash [40]. Cutaneous side effects of baricitinib and the other Janus kinase inhibitors include urticaria, rashes and palmoplantar pustulosis-like eruption. An overview of the drugs and potential skin lesions is shown in **Table 2**.

Other medications that can affect the skin and are currently of decreased importance in managing COVID-19 are interferon, oseltamivir, colchicine, azithromycin, and antimalarials.

As can be shown, multiple medications administered to patients with COVID-19 may affect the emergence of skin lesions, which may complicate the differential diagnosis between lesions caused by treatment and those caused by SARS-CoV-2 infection.

Finally, an important aspect is the effect of COVID-19 vaccination on the onset or exacerbation of skin diseases. Thus, reports exist on new cases of lichen planus and exacerbation of symptoms of this disease. The underlying mechanism needs to be clarified. So far, reports in the literature indicate that vaccination affects the induction of Th1 cell responses and, consequently, the secretion of cytokines that may cause the development of this disease [45]. For a detailed explanation of the expression of the immune system after vaccination against COVID-19, an example described in the context of the development of lymphomas will be used. The exact mechanisms of T-cell lymphomas induced by mRNA vaccines against COVID-19 are still unknown. However, in this case, it is also considered that these vaccines may have the ability to stimulate the immune system and thus over-activate immune responses. However, there are reports that mRNA vaccines against COVID-19 induce continuous stimulation of T and B cells, which may cause a high inflammatory response.

Consequently, this may lead to lymphoma or accelerate its progression [46]. It is worth emphasising that exacerbation of AD after COVID-19 vaccination was not observed, and neither was there any such correlation when patients were treated with biotechnology drugs such as sarilumab. In addition, no correlation has been found between the specific type of COVID-19 vaccine and AD exacerbation. Post-vaccination flare-ups of AD symptoms such as pruritus (usually mild and temporary) have been observed; however, no significant adverse effects have been noticed [45,47].

Pressure ulcers in COVID-19

Pressure injuries are among the most common aftermaths of prolonged confinement to bed. It is estimated that around 33.6% of patients with COVID-19 in a prone position develop pressure ulcers. On the other hand, pressure injuries in patients in a supine position tend to be rarer because they appear only in around 12% [48]. Among the iatrogenic risk factors for the appearance of pressure ulcers are the use of over two vasopressors, long-term mechanical ventilation, and the need to spend over two days in bed with

Table 2. Drug-induced skin lesions.

Drug	Possible skin lesions
Systemic glucocorticosteroids	Pruritus, erythema, urticaria, purpura
Low molecular weight heparin (LMWH)	Necrosis, erythema
Antiretrovirals (e.g. ritanavir)	Maculopapular rash, Exfoliative erythroderma, Stevens-Johnson syndrome
Tocilizumab	Pruritus, Psoriasiform dermatitis, urticaria
Anakinra	Generalized urticarial rash
Janus kinase inhibitors	Urticaria, rash

a pressure redistribution system [49]. Comorbidities strongly associated with the development of pressure injuries include hypertension, type-2 diabetes and obstructive lung diseases, e.g. asthma and COPD — furthermore, both patients with low BMI and severely obese are more likely to manifest pressure injuries. What is additionally crucial is the fact that COVID-19 itself may be a risk factor for developing pressure injuries [50]. It is based on a few factors. The first of them is low oxygen saturation on arrival at the hospital. It leads to local ischemia, which accelerates ulcer pain due to the accumulation of metabolites such as lactic acid [51]. Another element of the development of ulcers and its prognosis in COVID-19 is cytokine storm. High concentrations of interleukins, especially interleukin-6, cause prolonged inflammation. This is also may lead to local ischemia and more muscular pain related to ulcers [52]. Endothelial dysfunction, described extensively previously, may also impact the emergence of bedsores. The next factor which may be associated with the risk of pressure ulcers is comorbidities admitted to the ICU. Patients with advanced COVID have additional illnesses such as coronary disease or diabetes [53]. These conditions increase the risk of pressure ulcers [54,55] and are adverse prognostic factors in COVID treatment [56,57].

Furthermore, prone positioning, also used in patients with COVID-19 disease, is linked to a 3-fold increase in pressure-related injuries compared with supine positioning [58]. The need for medical equipment such as ECMO (extracorporeal membrane oxygenation) cannulas and endotracheal tubes is also a significant factor in increasing the risk of developing bedsores and other skin lesions. The skin at the site of insertions is vulnerable to infection and mechanical injury. In addition, pressure on the skin caused by passing cannulas may also lead to skin damage. Examples of ECMO injuries and pressure sores are presented in **Figures 4** and **5**.

Together, the elements mentioned above result in the necessity of additional care for patients with COVID-19. However, pressure ulcers are very challenging conditions. Treatment costs are incredibly high, forcing medical equipment to introduce prevention in the first place. It includes the rotation of patients, usage of pressure redistribution points in medical devices and additional strategies [60]. However, studies show that even intensive care personnel must learn about prevention methods for pressure ulcers [61]. This should concern officials in improving education and searching for more straightforward prevention techniques. Among them, one very promising is the usage of dressings in the form of foam and hydrocolloids. They were proven to be very effective and also relatively cheap [62].

Furthermore, studies of hydrogels based on zwitterion suggest they may also be used to treat pressure ulcers [63]. Another potential treatment for pressure injuries is heparin. Heparin is proven to be an effective drug in the prevention of hypoxia in COVID-19 [64], which was already mentioned as one of the most essential factors of pressure ulcers. However, there are reports that usage of heparin may not affect hypoxia in patients admitted to ICU [65].

Finally, one of the aftermaths of pressure ulcers is facial scars. As of the first half of 2022, there are not many reports about the frequency of this condition. However, first-case reports show that this may be a crucial problem in the following years [66].

Skin Failure in COVID-19

Another critical skin condition appearing in ICUs is skin failure. It is defined as the loss of integument associated with hemodynamic instability and/or additional organ failure [67]. The. The pathophysiology of this process is complex and should differ from pressure ulcers. Skin failure may occur independently from pressure ulcers or as a complication [68]. The most critical factors contributing to integumentary breakdown are hypoperfusion combined with hypothesised destruction of autosomes (i.e. areas of skin vascularised by one artery) and severe organ dysfunction contributed to other illnesses (e.g. myocardial infarction, shock, cerebrovascular incidents, etc.). Additionally, skin failures can be divided into two categories: acute, which most commonly occurs in ICUs and end-of-life, which mostly happens among terminally chronically ill patients [69].

Similarly to pressure ulcers, there is a strong relationship between COVID-19 and skin failure [70]. Besides the mechanisms mentioned above

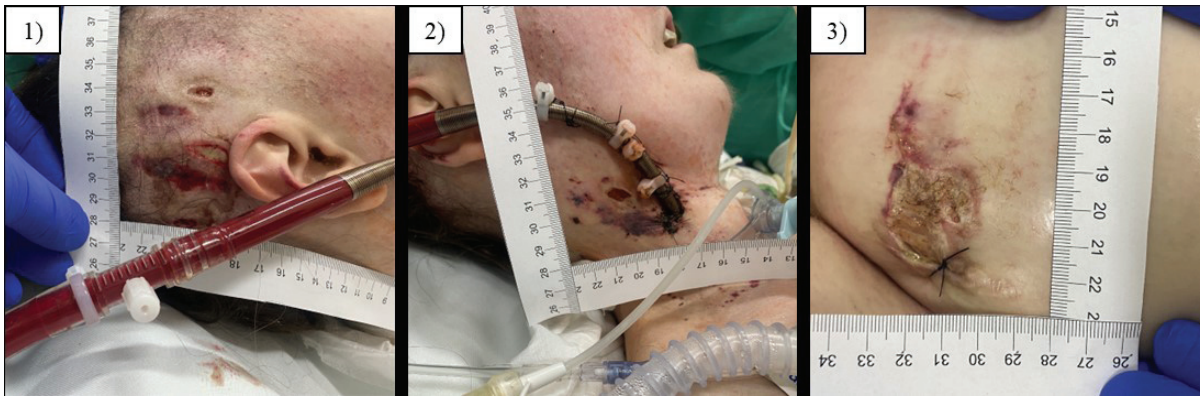

Figure 4. ECMO-induced skin lesions: 1) pressure ulcers in the area of the head resulting from the position of the VV ECMO cannula; 2) skin lesions around the cannula of VV ECMO with sores on the auricle; 3) skin lesion after removal of VV ECMO cannula in the groin area.

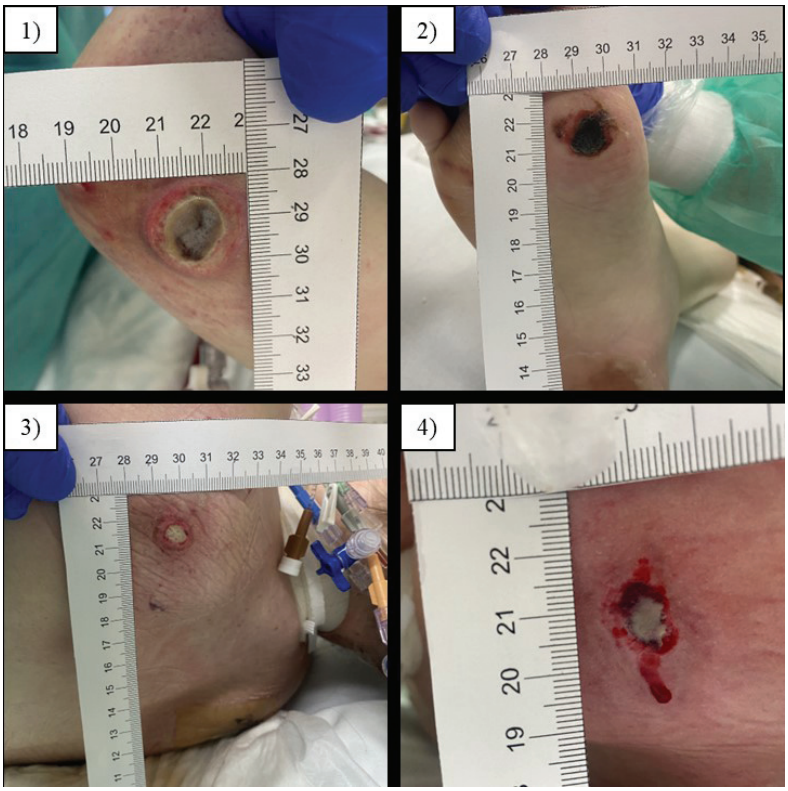

Figure 5. Pressure ulcer located in 1) the elbow region, 2) the sole, 3) the scapular region, and 4) the scapular region.

in pressure ulcers, there are also unique factors of COVID-19 that contribute to the development of skin failure. The first of them is vasculitis, which attacks small vessels of the skin. It is one of the most common complications both in COVID-19 and ICUs [71,72].

Contrary to Kawasaki-like, cutaneous vasculitis is common among adult patients [73]. Other risk factors for developing skin failure in COVID-19 are coagulopathy and complement-related microthrombosis. These are responsible for skin ischaemia and critical organ failures [27]. Furthermore, it is worth emphasising that the appearance of microthrombi might be associated with COVID-19, and their occurrence may be associated with an acute disease [74].

Unfortunately, it is hard to estimate the exact number of patients with skin failures because it is difficult to differentiate them from other skin conditions in ICUs.

Understaffing and quality of care

The hypothetical pathomechanism of decubitus ulcer formation is based on systemic processes leading to skin ischemia [75]. However, it is worth noting that other determinants of pressure ulcer problems include staff deficiency, worker exhaustion, and the practice of bedsores prophylaxis.

Medical personnel, including nurses, physiotherapists, and other medical professionals, have a significant role in preventing pressure sores [61].

The COVID-19 pandemic impacted previously underfunded health care, which resulted in shortages, especially at the beginning of the COVID-19 pandemic, not only of personal protective equipment for medical personnel but also a shortage of hospitals and hospital beds [76,77].

In addition, SARS-CoV-2 has shed light on medical staffing deficiencies that had already occurred before its onset [77].

During the COVID-19 pandemic, additional factors affected the reduction in the number of medical personnel; for example, in Poland, due to the restriction of work to one workplace, increased worker morbidity from COVID-19 at the peak of the pandemic, employee rotations, and retirement [77].

Personnel deficiencies, in particular, could impact the development of pressure sores, even more so in pronated patients, since it takes 4–6 workers to reposition a patient each time [58]. Moreover, patients with severe courses of COVID-19 require special care due to their condition and the number of medical procedures performed, resulting in greater staffing requirements.

Studies conducted in the USA, Canada, and some European countries [78] have shown that adequate nursing staffing affects hospitalised patients' outcomes and the shorter duration of hospital stays. Furthermore, the staffing level of nurses in hospitals is a determinant of the quality of nursing care [78].

Preventing pressure sores requires interdisciplinary collaboration, but the care process is critical.

Due to the hiring of volunteers, soldiers, medical students, and immigrants without waiting for the formalities of nostrification, calling people retired, and the insufficient time to educate newcomers, it is not sure whether these workers were adequately prepared to prevent pressure injuries [77,79]. It is essential to emphasise that knowledge regarding avoiding and caring for pressure ulcers is specialised. Thus, it is questionable that in such a limited period, with a deficiency of staff, training in this area would be provided sufficiently, especially since it has been shown that even ICU staff may have inadequate cognisance regarding methods of preventing bedsores [61].

Another aspect that could affect the development of decubitus ulcers is the inability of family members or relatives to be involved in hygiene routines, including preventive care for pressure ulcers, due to restrictions or prohibitions on visiting healthcare facilities.

Skin biopsy as a diagnostic method

Analysis and evaluation of skin lesions extended by tissue examinations can simplify the diagnosis and prognosis of patients infected with SARS-CoV-2. Cutaneous punch biopsy proposed by Laurence et al. appears to be a practicable and well-promising diagnostic instrument for detecting COVID-19 incidence and predicting the course of the disease [74]. Although the study was conducted on a small number of subjects (15 with severe/critical COVID-19 and 6 with mild/moderate COVID-19), the results are nevertheless promising. In their study, the severity of COVID-19 correlated with microthrombi. They observed the presence of microthrombi in patients with severe COVID-19, while these were undetectable in samples from subjects with a mild or moderate course. The formation of microclots may lead to cutaneous minification. The above indicates that skin lesions and their evaluation with tissue examination could help assess the condition of a patient with COVID-19 before severe symptoms develop, identifying patients at high risk of acute disease and, consequently, may allow for earlier implementation of appropriate treatment.

Discussion

As time passes and more cases are analysed, knowledge of the disease caused by SARS-CoV-2 is expanding.

Skin lesions are observed in patients during COVID-19 and are increasingly described.

It should be kept in mind that some of the observed lesions may be due to pharmacotherapy. To differentiate between lesions induced during the disease and those resulting from the implemented treatment, it would be necessary to discontinue the drugs suspected of causing skin lesions, which would entail discontinuation. In patients hospitalised for SARS-CoV-2 infection, multiple drugs are used simultaneously. Thus, in the case of an adverse skin reaction, it seems essential to consult a consultant allergist for treatment of acute skin symptoms and a possible diagnosis of hyperresponsiveness to the medications.

Finding the cause may contribute to the proper treatment of skin lesions; therefore, it seems necessary to consider implementing dermatological and allergological consultations.

Cutaneous manifestations may also be the single symptom of COVID-19, which may facilitate the diagnosis of this disease. The mechanism of skin lesions is unclear because it is difficult to determine their cause. They may result from a superposition of several factors, such as coagulopathy, other viral infections, or medication-related dermatoses. It seems necessary to extend the diagnostics of skin lesions during COVID-19 in order to understand their pathogenesis. Inadequate care due to staff shortages or inadequate education may lead to the development of pressure sores. Implementing solutions that could protect both patients and staff in the event of future pandemics would seem imperative.

The compilation of skin lesions presented in this paper may be useful in diagnosing COVID-19, indicating the need for testing for SARS-CoV-2 in the absence of other symptoms, early diagnosis of lesions, and patient prognosis for some of the lesions described.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Mechanisms behind corticosteroid resistance in obesity-induced airway inflammation – a review

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ABSTRACT

Obese non-eosinophilic asthma is defined as more severe asthma with severe symptoms, moderate airway hyperresponsiveness, elevated blood neutrophils, elevated biomarkers of non-type-2 inflammation, and low responsiveness to inhaled corticosteroids. Increased BMI is associated with a faster decline of FEV1 and FVC in adult asthmatics. The increased leptin concentration is connected with asthma by its ability to induce airway remodelling. Obesity-associated airway hyperresponsiveness is possibly mediated by NLRP3 inflammasome, IL-1β, and ILC3 cells. Increased sputum expression of NLRP3 and IL-1β is linked with increased neutrophil numbers, airflow obstruction, and worse asthma control. Accumulation of proinflammatory cytokines like IL-17, IL-1β, TNF-α, and reactive oxygen and nitrogen species contributes to corticosteroid resistance in obese asthmatics. The processes on the cellular level leading to steroid hyporesponsiveness include a reduced level of glucocorticoid receptor (GR) isoform – GR-α, the dysregulation of the isoforms concentration GR-α/GR-β, increased phosphorylation at Ser 226, and decreased expression of histone deacetylase 2. The best way to improve sensitivity to corticosteroids in this patient group is weight loss. Bariatric surgery is the most effective solution. However, patients may find it beneficial to implement lifestyle changes or to use GLP-1 analogues. Identifying underlying mechanisms of resistance to corticosteroids in obese asthmatics will allow for more effective asthma treatment in the future and could lead to long-term reduction of treatment costs.

Introduction

Asthma is described by The Global Initiative for Asthma (GINA) as a heterogeneous disease characterised by chronic airway inflammation and a history of intense, varying over time symptoms such as wheezing, coughing, tightness in the chest, and shortness of breath with expiratory

airflow limitation [1]. Severe asthma refers to individuals who cannot achieve good control despite high doses of optimal medication. The European Respiratory Society, as well as the American Thoracic Society Task Force, have revised the definition of severe asthma to include asthma that requires step 4–5 asthma treatment protocol according to GINA (high-dose inhaled corticosteroids (ICs) and LABA or leukotriene modifier), or systemic corticosteroids for over 50% of the previous year to prevent it from being uncontrolled or remained uncontrolled despite this therapy [2]. In severe asthma, a higher body mass index (BMI) is an aggravating factor in disease control, and obese patients often require higher doses of ICS [2]. BMI correlates with the risk of new asthma onset in men and women [3]. According to the British Thoracic Society Difficult Asthma Registry, 48% of adult severe asthmatics are obese compared to a 25% prevalence of obesity in the general British adult population [4]. This may result from the obesogenic effects of systemic corticosteroids used in this population [3]. Obese adults are at higher risk of asthma exacerbations and mechanical ventilation than lean adults [3]. Cluster analysis applied on a large cohort defined cluster "obese non-eosinophilic asthma" as late-onset, of predominantly female sex, high symptoms, low atopy, low sputum eosinophils, moderate airway hyperresponsiveness, reversibility of obstruction, and low responsiveness to inhaled corticosteroids [5]. A chronic low-grade systemic inflammatory state is characteristic of obesity, and the levels of C-reactive protein (CRP) and IL-6 are elevated in obese asthmatics, which corresponds with neutrophilic airway inflammation [6]. This observation aligns with the fact that obese asthmatics have reduced responses to conventional asthma treatment. A study on adult-onset obese asthma patients revealed that severe obesity, elevated blood neutrophils, and elevated biomarkers typical for T2-low inflammation are associated with more severe asthma [7]. T2-low airway inflammation is characterised by airway remodelling and poor anti-inflammatory response. Its immunopathogenesis involves intrinsic neutrophil abnormalities, inflammasome pathway activation, and IL-17 pathway activation [2]. Obesity significantly affects asthma patients' corticosteroid resistance, with its plasma concentration after oral administration negatively correlated with BMI and prednisone clearance being positively correlated with BMI [8]. Therefore, to control their asthma symptoms, obese asthmatics are prescribed higher doses of corticosteroids, which leads to iatrogenic side effects and increased healthcare costs. The relation between asthma and obesity underlines the importance of understanding the mechanisms behind corticosteroid resistance in obese asthmatics. It may allow us to find a more effective treatment and improve asthma control.

Decreased pulmonary function

Monitoring pulmonary function, especially forced expiratory volume in 1 second (FEV1), is crucial for asthma control since it reflects airway obstruction, the essence of asthma pathophysiology [9]. Decreased FEV1 is a significant sign of severe asthma and has been correlated with increased BMI. Another parameter that obesity affects is forced vital capacity (FVC), which may result from the accumulation of adipose tissue, limiting the mobility of the thorax and diaphragm. A 2022 meta-analysis found that BMI was inversely related to pulmonary function parameters FEV1 and FVC. Additionally, increased BMI was associated with a faster decline of FEV1 and FVC in adult asthmatics with a BMI greater than 25 kg/m2 [10,11]. However, this obesity-associated phenomenon was not observed in patients without asthma [11]. Reduced FEV1 in obese asthmatics could result from airway synapsis, a physiological imbalance between the calibre of the airways, and the expansion of lung parenchyma [12]. Increased BMI was associated with dysanapsis in children with and without asthma. Additionally, dysanapsis in obese asthmatics is related to severe exacerbations and higher systemic steroid use [12]. The processes behind decreased pulmonary function in obese asthmatics remain unclear, necessitating further investigation beyond the direct physical impact of obesity [9].

Obesity and asthma: adipokine influence on the course of the disease

Obesity alters the inflammatory system regulation, which tends to cause an imbalance between

the pro-inflammatory and anti-inflammatory markers. Accumulation of excessive fat in obese patients results in excess macronutrients in the adipose tissues. Obesity promotes the release of proinflammatory mediators like leptin, resistin [13], Tumor necrosis factor α (TNF-α), IL-6 [14] and interleukin 1 (IL-1) family (including IL-1β [15] and IL-18 [16]) [17,18]. It is suggested that the imbalance between the pro-inflammatory and anti-inflammatory cascade in obese individuals increases the risk of asthma and its severity [19]. Behind the development of obesity lies an increased triglyceride supply, leading to hyperplasia and hypertrophy of the adipose tissue [20]. Hypertrophied adipocytes secrete proinflammatory mediators affecting macrophage activity, further releasing pro-inflammatory cytokines. In addition, the adipocyte experiences hypoxia and accumulation of cytotoxic molecules mediating cellular stress and exacerbating the local inflammatory response [21,22,23]. Obese patients have higher levels of leptin, associated with airway hyperactivity, and an increase in the expression of other pro-inflammatory markers such as TNF-α and IL-6 [24,25,26]. A 2023 study by Wanatabe et al. showed that the building up leptin-producing monocytes might be involved in asthma pathogenesis [27]. A Zhigang Tian study showed that leptin modulates the immune system functions, including activation, differentiation, stimulation of proliferation, and activation of immune cells such as macrophages and natural killer cells [28]. Based on a review article by Matarese et al., leptin polarises helper T cell cytokine production towards a proinflammatory phenotype (Th1 cells producing Interferon-gamma, IL-2) while inhibiting the production of anti-inflammatory cytokines (Th2 cells producing IL-4) which as a result induces inflammatory response and activation of monocytes, CD4+, and CD8+ T cells [29]. Previous studies in overweight patients demonstrated that single nucleotide polymorphisms (SNPs) of leptin (LEP) and adiponectin (ADIPOQ) gene sequences are modified, potentially losing their shielding effect against atopy and asthma instigation. Obesity-induced LEP and ADIPOQ gene modifications are associated with a higher risk of the development of obese asthma phenotype [30]. Leptin plays a significant role in respiratory and lung function, and the expression of leptin receptors in bronchial epithelial cells and lung fibroblasts relates to airway remodelling in asthma [31]. Leptin-induced bronchial remodelling is characterised by goblet cell metaplasia, smooth muscle hypertrophy, increased angiogenesis and airway epithelial cell hypersecretion [32]. Additionally, obesity promotes low levels of anti-inflammatory adipokines, such as adiponectin, which acts as a leptin antagonist. Adiponectin has down-regulating effects on the inflammatory system as it counteracts leptin's effects by inhibiting eosinophil recruitment, further promoting inflammatory response in obese patients [33].

NLRP3 inflammasome–IL-1β–ILC3 axis

Kim et al. proposed that obesity-associated asthma and airway hyperresponsiveness may be caused by inflammation mediated by components of the innate immune response – NLRP3 inflammasome, IL-1β, and ILC3 cells, which are activated in the lungs during nutritional excess [34]. Pattern recognition receptors (PRRs) expressed by macrophages, monocytes, dendric cells, neutrophils, and epithelial cells play a crucial role in activating this innate immune response. PRRs include the membrane-bound Toll-like receptors (TLRs) and cytosolic PRRs like Nod-like receptors (NLRs). PRRs can induce an innate immune response by activating inflammasomes – intracellular oligomeric complexes present predominantly in immune and epithelial cells [35]. The NLRP3 inflammasome constitutes pro-caspase-1, an apoptosis-associated speck-like protein containing CARD (ASC) adaptor protein and nucleotide oligomerisation domain-like receptor protein 3 (NLRP3). Two separate processes are needed for the activation and assembly of NLRP3 inflammasome. First, pathogen-associated molecular patterns (PAMPs) are identified by PRRs, such as Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4). Activation of assembled inflammasome requires a second signal, provided by danger-associated molecular patterns (DAMPs) like extracellular ATP, potassium efflux, and monosodium urate crystals. The NLRP3 inflammasome activation promotes the autocatalysis of pro-caspase and the activation of caspase 1, which converts pro-IL-1β and pro-IL-18 into active forms [36,35,37,38]. Simpson

et al. discovered that the sputum macrophages of neutrophilic asthmatics exhibit higher expression of TLR2, TLR4, NLRP3, caspase-1, and IL-1β [31]. Higher sputum expression of NLRP3 and IL-1β was associated with increased sputum neutrophil numbers, airflow obstruction, and worse asthma control in asthmatics treated with ICs [40]. The high intake of saturated fatty acids (SFAs) is an independent risk factor for asthma development, and high plasma levels of SFAs after a high-fat meal are associated with higher sputum neutrophil percentages. SFAs bind to PRRs, mainly TLR4, triggering proinflammatory processes like the assembly of the NLRP3 inflammasome, activation of caspase-1, and IL-1β release [41]. Epithelial barrier function may be affected by IL-1β, and mucin expression may be elevated by IL-1β and IL-17a stimulation, indicating a possible connection between enhanced inflammasome expression and impaired barrier function [42]. Neutrophil-predominant asthma may be driven by IL-17 produced by TH17 lymphoid cells, which may also be involved in corticosteroid resistance [44]. IL-1β could be essential for regulating IL-17 lung production – blocking the IL-1 signalling by IL-1R antagonist – anakinra led to abolishing the IL-17 pathway [34]. Group 3 Innate lymphoid cells (ILC3s) can mimic the function of Th17 cells, and it was suggested that the production of IL-17 by ILC3s might be a significant contributor to airway neutrophilic inflammation [34]. Unfortunately, a phase II study using a human anti-IL-17 receptor monoclonal antibody (brodulimab) in subjects with moderate and severe asthma did not show a therapeutic effect [44]. In obese mice, the obesity-induced airway inflammation and airway hyperresponsiveness are driven by IL-1β and mediated by an expanded population of pulmonary IL-17+ ILC3 cells. ILC3s produce neutrophil chemoattractants like TNF-α, Granulocyte-mac-

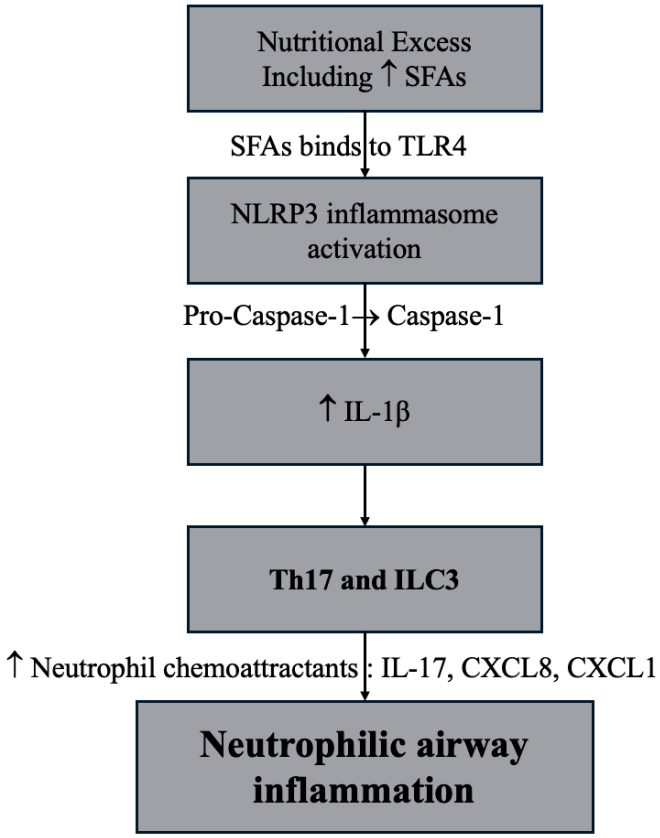

Figure 1. Processes leading to airway inflammation in obese asthmatics. SFAs- saturated fatty acids; TLR4- toll-like receptor 4; NLRP3 – nucleotide oligomerization domain-like receptor protein 3; Th17 – Th17 lymphoid cells; ILC3 – innate lymphoid cells 3; SFAs bind to pattern receptors like TLR4, triggering the assembly and activation of the NLRP3 inflammasome. This promotes the activation of caspase-1 and the release of IL-1β by M1 lung macrophages. IL-1β stimulates Th17 lymphocytes and ILC3 to produce neutrophil chemoattractants like CXCL1, CXCL8, and IL-17, which leads to airway neutrophilic inflammation.

rophage colony-stimulating factor (GM-CSF), chemokine (C-X-C motif) ligand 8 (CXCL8, also known as interleukine-8), and chemokine (C-X-C motif) ligand 1(CXCL1) after IL-1β stimulation [45]. Dexamethasone did not affect the expression of CXCL8 and CXCL1 in ILC3s after IL-1β stimulation, indicating that the IL-1β-ILC3-CXCL8/CXCL1 axis may be associated with corticosteroid resistance and neutrophilic inflammation which are the characteristics of adult-onset obese asthmatics [41]. In a mouse model of severe asthma, a highly specific NLRP3 inhibitor called MCC950 decreased the production of IL-1β and the release of Th2 cytokines and chemokines, preventing airway hyperresponsiveness and decreasing asthma inflammation [46]. The results of another study showed that MCC950 can successfully lower NLRP3 inflammasome-mediated IL-1β release from peripheral blood mononuclear cells (PBMCs) from asthma patients as well as healthy subjects, and the most significant effects occurred in PBMCs from severe asthmatics [47]. This study demonstrated the therapeutic potential for NLRP3 inflammasome inhibition in clinical settings and may represent a new management approach in severe, T2-low asthma. The impact of nutritional excess in obese asthmatics on innate immune response is illustrated in **Figure 1**.

Alterations of glucocorticoid receptor function in obesity-related asthma leading to corticosteroid resistance

Khali et al. describe two types of corticosteroid resistance among asthmatics. Type 1 – cytokine-induced is associated with increased production of certain cytokines and chronic corticosteroid exposure, and Type 2 – primary cortisol resistance is connected with mutations in the glucocorticoid receptor (GR) gene [37]. Glucocorticoid action in cells is mediated by a specific receptor protein, the glucocorticoid receptor (GR). It is expressed in almost all human tissues and organs [48,49]. Alternative splicing of pre-mRNA generates two isoforms of the human glucocorticoid receptor- GR-α and GR-β. GR-α (active isoform of GR) mediates glucocorticoid action, and GR-β (innate isoform of GR) acts as its dominant inhibitor and is unable to bind steroids [48,49]. A body of literature has shown that one of the main causes of steroid resistance in severe asthma is the defect in GR-α and dysregulation of the GR-α/GR-β ratio [41,48,49]. According to some studies, IL-17 cytokines are up-regulating GR-β and down-regulating GR-α [50,51]. Zijlstra et al. showed that reduced glucocorticoid sensitivity positively correlates with neutrophilic airway inflammation [49]. Th17 lymphocytes play a major role in the induction of neutrophilic airway inflammation. Recently, scientists have indicated that Th-17 cytokines such as IL-17A and IL-17F can be an important response mediator to glucocorticoid treatment [50,51]. In respiratory epithelial cells, IL-17A and IL-17F induced the expression of GRβ, which mitigated GRα's anti-inflammatory effect and increased corticosteroid resistance [52]. High levels of IL-17 cytokines have been reported in chronic inflammatory disorders such as obesity. It may lead to steroid resistance in obese individuals with asthma [49–51,53]. A study by Kim et al. revealed that increased NLRP3 inflammasome/IL1-β activation significantly contributed to corticosteroid resistance [34]. This effect may be explained by the fact that IL-1β modulates the TH17 cell differentiation and IL-17 production, and asthmatics resistant to corticosteroids showed increased Th17 cell counts and levels of IL-17A [54]. GRs recruit histone deacetylase 2 (HDAC2) to mediate their anti-inflammatory activity as a key transcriptional co-repressor [55,56]. Studies have shown that the expression of HDAC2 is reduced in severe asthma [57]. The reduced expression of HDAC2 is an effect of increased oxidative and nitrative stress, which leads to the nitration of HDAC2 and its degradation, ubiquitination, and inactivation [48,58]. Obesity-related metabolic dysfunction causes increased oxidant production by activated airway epithelial cells from oxidative and nitrosative bursts [59]. Uncoupling of the NOS (nitric oxide synthase) is a potential mechanism behind increased oxidant production in airway epithelial cells [60]. Arginase is an enzyme that metabolises the L-arginine (substrate for NOS) to L-ornithine and urea [61]. Asthmatics present with increased arginase expression, leading to reduced L-arginine availability for NOS [61]. This, in turn, can lead to an increased level of endogenous NOS inhibitor – asymmetric dimethylarginine, causing enhanced production of reactive nitrogen and

oxygen species in airway epithelial cells. All those changes can result in the inactivation of HDAC2 and reduced response to corticosteroids [62]. Furthermore, the glucocorticoid action may be alternated by the phosphorylation status of GR [48]. Phosphorylation at Ser211 enhances GR activity, and phosphorylation at Ser226 has an inhibitory effect [48,49]. Other studies have suggested that an imbalance between the phosphorylation of Ser211 and Ser226 of GR, which is regulated by MKP (Mitogen-Activated Protein Kinase Phosphatases), may contribute significantly to steroid resistance in obese asthmatics [49]. MKP is an anti-inflammatory marker of glucocorticoid-induced activation. Obese asthmatics with poor response to steroids have significantly reduced MKP expression. Induction of MKP by glucocorticoids may be inhibited by increased release of pro-inflammatory cytokines associated with obesity. MKP is a phosphatase that inactivates p38 MAPK (p38 mitogen-activated protein kinase) via dephosphorylation [25,49,63]. Increased level of p38 MAPK was observed in peripheral blood mononuclear cells and bronchoalveolar lavage in obese asthmatics. It leads to the phosphorylation of GR at Ser226, which inhibits transcriptional activity, promotes nuclear export, and has an inhibitory effect on GR function [41]. Additionally, mediated by NLRP3 inflammasome release of IL-1β could induce Ser226 phosphorylation, which may increase steroid hyporesponsiveness neutrophilic asthma [40,41]. Described mechanisms behind corticosteroid resistance in obese asthmatics are illustrated in **Figure 2**. Lea et al. highlighted that inhibitors of p38 MAPK can help restore corticosteroid sensitivity in peripheral blood mononuclear cells of patients with severe asthma [55]. Clinical trials showed the beneficial effects of using p38 MAPK inhibitors in chronic obstructive pulmonary diseases, with the levels of inflammatory biomarkers significantly reduced in two clinical trials [65,66]. TNF-α secreted by macrophages increases the sputum neutrophilia and induces airway hyperresponsiveness. A study by Jiang et al. showed that TNF-α significantly increased TNF-α and IL-6 mRNA expression and decreased mRNA and protein levels of GRα via NF-κB and p38 MAPK signalling pathways in nasal epithelial

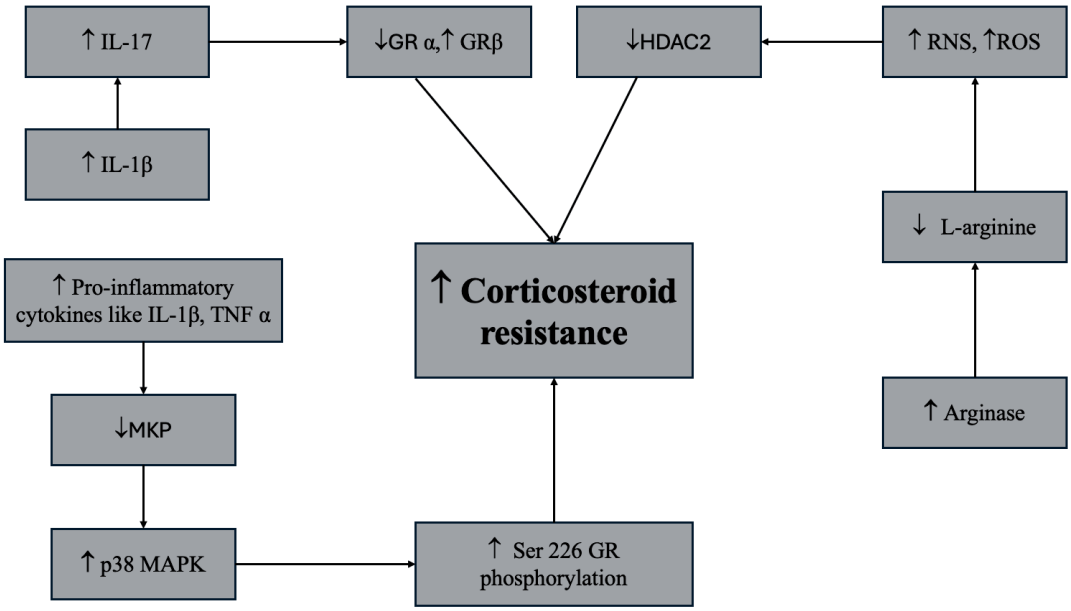

Figure 2. Mechanisms behind corticosteroid resistance in obese asthmatics. GR-glucocorticoid receptor; GR-α – α isoform of glucocorticoid receptor, GR-β – β isoform of glucocorticoid receptor and down-regulating GR-α; HDAC2-Histone deacetylase 2; RNS – reactive nitrogen species; ROS- reactive oxygen species; TNF-α-Tumor necrosis factor α MKP Mitogen-Activated Protein Kinase Phosphatases; p38 MAPK – p38 mitogen-activated protein kinase. IL-1β stimulates Th17 lymphoid cells to increase the release of IL-17. IL-17 cytokines down-regulate active GR-α and upregulate innate GR-β isoform; Higher expression of arginase leads to lower levels of L-arginine – substrate for nitric oxide synthase. Those changes lead to increased production of RNS and ROS. Increased oxidative stress results in lower expression of HDAC2 and reduced GR-mediated anti-inflammatory activity; Reduced expression of MKP results from the accumulation of pro-inflammatory cytokines like TNF-α and IL-1β. The result of lower expression of MKP is an increased level of p38 MAPK, which leads to the GR phosphorylation at Ser226- which has an inhibitory effect on GR.

cells of patients with chronic rhinosinusitis, leading to reduced corticosteroid response [67]. The use of golimumab (another anti-TNF-α drug) in severe asthma was terminated after the phase 2 study due to the increased risk of severe infections [68]. Holgate et al. failed to demonstrate the clinical efficacy of etanercept in patients with severe asthma. However, no unexpected safety findings were observed [69]. Future research must evaluate the long-term benefit profile of anti-TNF-α medication in severe neutrophilic asthma.

Bariatric surgery as a way to lose weight and increase asthma control

Post-bariatric surgery patients have a more significant weight loss (22–36%) compared to patients who received non-surgical treatment (4.1–14.2% weight loss) [70]. Post-bariatric surgery weight loss improves airway responsiveness, asthma control, lung function, and the quality of life of obese asthmatics, reducing exacerbations and hospitalisations [18,71,72]. Guerron et al. showed that as early as 30 days post-bariatric surgery, the number of prescribed asthma medications was 27% lower than the pre-operation. The reduction was progressive over time, and asthma medication use was lower by 48% two years after surgery than the pre-operative average. There was no significant difference in the reduction of asthma medication use depending on the type of surgery (Sleeve gastrectomy, Adjustable gastric band, Duodenal switch, Roux-en-Y gastric bypass), and all bariatric procedures showed a similar pattern [71]. The concentration of pro-inflammatory markers TNF-α, leptin, IL-6, IL-8, and IFN-gamma has decreased with weight loss in obese asthmatic patients after bariatric surgery [18,73]. The underlying mechanisms for these improvements must be elucidated. Womble et al. suggested that vertical sleeve gastrectomy in mice would improve glucose tolerance, airway inflammation, resistance, and fibrosis induced by obesity and chronic allergen challenge [74]. Research has also shown that bariatric surgery can influence the gut microbiome [75], which has been studied for its role in obesity-induced conditions like asthma. Obesity increases proinflammatory molecules in the blood and alters the gut microbiome, with a decreased abundance of *Akkermansia muciniphila* directly associated with asthma severity [76]. By raising the gastrointestinal pH, bariatric surgery induces a substantial shift in the gut microbiome, with increased *Akkermansia muciniphila* abundance at three months and sustained through 12 months [9,75]. The positive effect of bariatric surgery on lung function in the obese population has also been reported. Nguyen et al. study found that obese patients after laparoscopic gastric surgery experienced an improvement of 12% in FEV1, 9% in FVC, 15% in peak expiratory flow (PEF), and 30% in forced expiratory volume at 25–75% of FVC after 12 months. The improvement occurred as early as three months post-intervention. The fraction of patients with an abnormal FEV1/FVC ratio (defined as less than 0.8) declined from 9.6% before surgery to 1.9% after surgery [77]. Due to the invasive character of bariatric surgery, the number of obese asthmatics with severe symptoms who can be operated on is limited.

Additionally, severe asthma increases the operational risk. Lifestyle changes, including diet and exercise interventions, are not as effective as bariatric surgery but might help more patients lose weight and positively impact asthma course [78]. A randomised study showed that even 5% weight loss is associated with improved asthma control and significant increases in FEV1 and FVC [78]. Scott et al. showed an association between gynoid fat reduction and neutrophilic inflammation in women. In contrast, in men, there is a correlation between reduced saturated fat consumption and absolute counts of sputum neutrophils [79]. Asthmatics in the diet group who improved asthma symptoms had significantly lower consumption of calories, carbohydrates, total fats, saturated fats, and polyunsaturated fatty acids (PUFA) [78]. Saturated fats and PUFA consumption lead to inflammatory responses [78]. Mediterranean Diet rich in antioxidants and cis-monounsaturated fatty acids was associated with reduced asthma symptoms in children and lung function improvement [80]. Increased physical activity augments weight loss, improves lung function, and has anti-inflammatory effects. Aerobic training reduces the fractional exhaled nitric oxide (FeNO) and serum levels of proinflammatory mediators like IL-4, IL-6, TNF-α, CCL2, and leptin and increases the levels of anti-inflammatory

cytokines like IL-10 and adiponectin [81]. Physical activity and caloric restriction improve the level of 25(OH)D by reducing visceral fat tissue and increasing the availability of fat-soluble vitamin D. Reduced serum levels of vitamin D are associated with worse asthma control and exacerbation [82].

GLP-1 analogues

Insulin resistance (IR) associated with obesity is an independent risk factor for adult asthma development. Studies have shown that IR in patients with asthma is related to decreased lung function and lower response to treatment with corticosteroids and β2-adrenergic agonists [83]. A group of medications widely used in type 2 diabetes known as glucagon-like peptide 1 (GLP-1) receptor agonists may also improve asthma control in obese asthmatics. They enhance glucose tolerance and are linked with weight reduction, downregulation of inflammatory response, and reduced cardiovascular risk in obese patients [84,85]. GLP-1 receptor agonists work similarly to the hormone GLP-1, which is primarily secreted by intestinal enteroendocrine L-cells in response to food intake. In patients with diabetes, GLP-1 receptor agonists increase insulin secretion and suppress glucagon release [85]. The potential mechanisms behind weight loss caused by GLP-1 analogues are appetite suppression, lowered food intake, slowdown of gastric emptying, and prolonged satiety [84]. The average weight loss varies depending on dosage, administration route, and drug type. For instance, studies on two agonists showed that the mean weight loss with liraglutide (administered daily, subcutaneously) was 4.8–7.2 kg [85], and with semaglutide (administered once a week, subcutaneously) was 10–15 kg [86]. Research performed on obese asthmatic murine model reported increased levels of GLP-1 receptors in lung epithelial cells, and administrations of GLP-1 receptor agonists reduced levels of neutrophils and, thereby, airway inflammation in those mice [87]. Foer et al. reported that patients with asthma and type 2 diabetes who received GLP-1 receptor agonists had fewer asthma exacerbations than patients treated with other medications [88]. Although the current results are promising, to obtain more convincing evidence, randomised, placebo-controlled trials addressing both obesity and asthma are required to evaluate the effectiveness of using GLP-1 receptor agonists in obese asthmatics.

The beneficial effect of azithromycin on severe asthma course

Studies have shown that low-dose azithromycin can effectively treat severe asthma [89,90]. Macrolide antibiotics have two essential properties: anti-bacterial and anti-inflammatory [90]. Gibson et al. conducted a study for over 48 weeks on 420 adult patients with severe asthma who were administered 500 mg of azithromycin two times a week. The number of exacerbations significantly decreased, and quality of life improved in the azithromycin-treated group compared to patients who were administered placebo [90]. Those effects were observed in both non-eosinophilic and eosinophilic types of asthma. Thomas et al. evaluated the long-term impact of azithromycin on asthma remission in patients with persistent, uncontrolled asthma. After 12 months in the azithromycin arm, the proportion of patients that achieved remission was significantly higher compared to the placebo arm (50.6% vs 38.9%; $p = 0.032$) [91]. Currently, an ongoing study will evaluate the effect of azithromycin on asthma exacerbations in obesity-induced asthma [92].

Further studies are needed to find out how azithromycin reduces the number of asthma exacerbations. Possible mechanisms include pulmonary bacterial clearance and stimulating both IL-6 and IL-17 pathways. In conclusion, azithromycin may be a practical addition to asthma treatment in patients who are most vulnerable to exacerbations and who cannot control asthma symptoms with appropriate inhaled therapy [90,93].

Conclusions

Obese asthmatics tend to present with more severe asthma, experience more exacerbations, and require higher doses of ICs or even need to use systemic steroids to achieve satisfactory asthma control. It may lead to iatrogenic side effects and higher medical costs, which underlines the importance of understanding the mech-

anisms behind steroid resistance in obese asthmatics. Unfortunately, most of the research is still theoretical, and further exploration is required to understand the mechanisms behind corticosteroid insensitivity in this patient group. Identifying underlying resistance mechanisms to corticosteroids in obese asthmatics will allow for more effective asthma treatment in the future and could lead to long-term reduction of treatment costs. Some promising therapies may improve disease control in obese asthmatics, including blocking IL-1β signalling, using anti-TNF-α-medications, or using anti-IL-17a antibodies; however, future research needs to evaluate the long-term benefit profile of anti-TNF-α medication in severe neutrophilic asthma. Currently, research suggests that the best way to improve asthma control and reduce the demand for the use of ICs in obese asthmatics is weight loss. Currently, bariatric surgery is the most effective solution. However, patients may find it beneficial to implement lifestyle changes or use GLP-1 analogues.

Methodology – Search strategy

Papers published between 2005 and 2023 were identified by PubMed literature searches using the terms: "obese asthma"; "NLRP3 inflammasome", "IL-1β"; "IL-17", "ILC3", "corticosteroid resistance"; "steroid-resistant asthma"; "glucocorticoid receptor ";" Histone deacetylase 2"; 'Mitogen-Activated Protein Kinase Phosphatases'; "bariatric surgery"; "GLP-1 analogues"; Additional publications were selected through the internet from the references of those papers. Only articles in English were considered.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

JMS Journal of Medical Science

Introducing bromine in the molecular structure as a good strategy to the drug design

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ABSTRACT

Nowadays, the search for new pharmaceuticals results in the development of thousands of new substances. One of the effective drug design strategies is to modify a previously obtained and studied substance. A very popular modification is the introduction of halogens into the structure of drugs, most often these are fluorine or chlorine atoms. However, the introduction of bromine into the structure of a potential drug also has a number of advantages. A good example would be natural substances extracted from marine organisms, which have been studied and proven to be effective in various diseases, including antibiotic therapy of resistant bacteria. Numerous studies justify the usage of bromine and its isotopes in therapy (both in diagnostic imaging and radiotherapy). To better explain the impact of "bromination," numerous researchers have described such a phenomenon as "halogen bond." Due to the presence of the so-called "sigma-hole" in the halogen atom of an organic molecule, it is possible to form these bonds, which results in a change in intermolecular and intramolecular interactions. Such changes can favorably affect drug-target interactions. The advantages of "bromination" include an increase in therapeutic activity, a beneficial effect on the metabolism of the drug and an increase in its duration of action. Besides, the phenomenon of heavy atom effect can be used to increase the effectiveness of photodynamic therapy and radiosensitization. Unfortunately, "bromination" is not without drawbacks, which we may include increased toxic effects and accumulation in the organism.

Introduction

Drug discovery strategies

From the beginning of pharmacotherapy to the present day, a huge number of drugs and substances with potential therapeutic effects have been developed. Since ancient times, products of natural origin, such as plants or animal products, have been used. With the advancement of science and the development of chemistry, the isolation of chemical compounds or their mixtures began. Even to this day, the searches for new drug-like substances often involve their discovery in natural sources. However, the main source of new potential drugs is their synthesis and development of previously unknown substances. Often these are modifications of already known drugs or molecules of natural origin to improve their therapeutic parameters, thus increasing their potential for treatment.

One of the strategies for finding new original drugs is to introduce new substituents into the molecule of an already known, studied drug. Substitution of new functional groups is always associated with a change in pharmacodynamic and pharmacokinetic properties. New moieties in the molecule often lead to an increase in therapeutic activity, reduction or complete elimination of side effects. Very often halogens are introduced into the structure of a drug. Excluding extended substituents and focusing on the percentage of individual atoms in the molecules of registered drugs, the FDA presented the following statistics [1].

It can be deduced from the diagram that halogens present the majority of structural modifications and are often chosen as a strategy for finding new therapeutics. Speaking of bromine, a relatively low level of interest among researchers is apparent compared to chlorine and fluorine. Nonetheless, the element appears among the top five heteroatoms in drugs used in disorders of the nervous, sensory and respiratory systems. This demonstrates its potential to pass through biological barriers including blood-brain (this subject will be expanded upon in later sections of the article). The history of bromine in medicine begins with ancient times.

History of the discovery of bromine

Molecular bromine was discovered by two scientists independently of each other. German chemist Carl Löwig isolated bromine from swamp water in 1825, and in 1826 French scientist Antoine Balard extracted bromine from marine algae remains [2,3]. Molecular bromine has strong disinfectant, irritant and oxidizing properties when in presence of organic molecules so it has no use in medicine. The simplest examples of bromine use are ammonium, sodium and potassium bromides. In the old days, these salts were used as sedatives. Due to a number of side effects, including so-called "bromism" and the existence of safer and more effec-

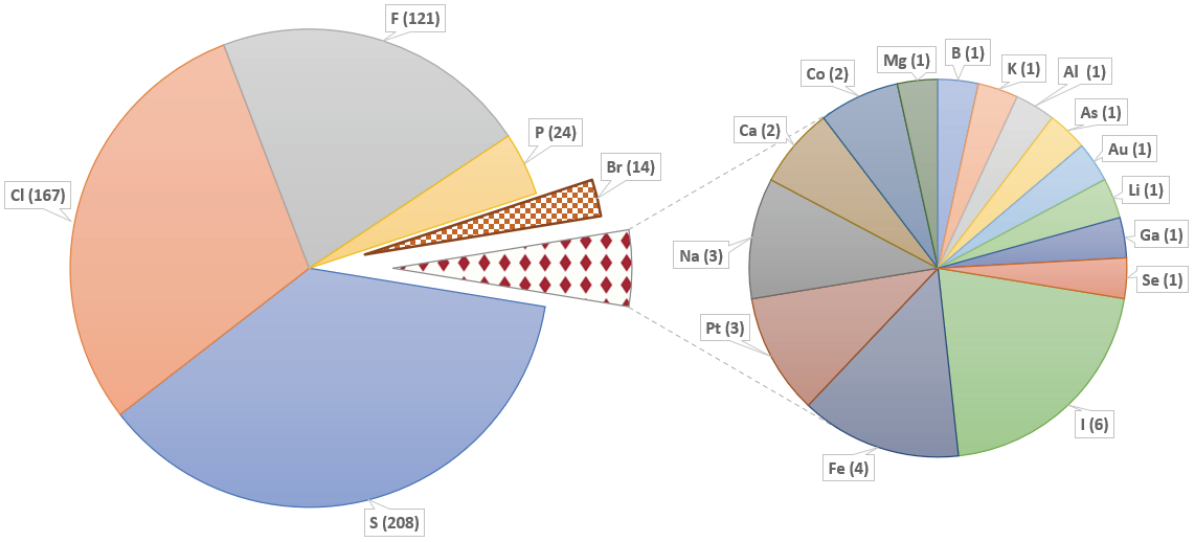


Figure 1. Occurrence of heteroatoms in medicinal substances (numbers in brackets mean the number of registered drugs reviewed in the paper) [1].

tive therapeutics, they fell out of use, although even today, they are still used in medicine in rare cases. Bromism is the consequence of bromide cumulation after long-term usage. The symptoms of this disease are anxiety, ataxia, psychosis, stupor, anorexia and rashes. But being incorporated into an organic molecule, it is devoid of these properties and can show a positive effect on the effectiveness in treating various diseases. The main sources of organic bromine derivatives are marine flora and fauna. Due to the high bromine content in seawater, these organisms produce metabolites, most of which contain a bromine atom in their structure. By 1999, more than 1600 compounds of this type had been isolated and identified. By 2020s another 12000 marine-derived compounds had been discovered, which not only expanded the list of potential drugs, but also served as inspiration for the design and synthesis of new derivatives. Moreover, it is estimated that every year the list of these compounds grows by several hundred more molecules [4].

Brominated indole derivatives – natural therapeutics with wide applications

A good example of potential therapeutics of natural origin are indole alkaloids. As mentioned above, due to the high halogen content of seawater, compounds from this source are often characterized by the presence of bromine in their structure. This group of metabolites is very varied and numerous studies indicate a potentially wide range of applications in medicine. For example, the following activities have been proven:

- Biocidal (including antibacterial, antifungal, antileishmanial, antiplasmodial, anti-HIV, larvicidal) [5–13];
- Opioid receptor agonist [14];
- Anti-inflammatory [15];
- Vasodilatory [16,17];
- Cholinesterase inhibitors [18];
- CB1 receptor inhibitors [19].

An interesting aspect of the therapeutic application of brominated indoles is their broad spectrum of activity against bacteria. As the problem of antibiotic resistance is increasing, the search for completely new structures seems to be crucial in solving this issue.

Weihong Wang et al. isolated from the tunicate species *Eudistoma sp.* 7 indole derivatives (**1–7**) (**Figure 2**) and tested their activity on selected bacterial strains (*Staphylococcus aureus* ATCC 6538, *Micrococcus lutes* ATCC 9341, *Staphylococcus epidermidis* ATCC12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 11775, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 4352). After conducting tests to determine MICs for the obtained compounds, the authors deduced that the most promising compound was **6**, which showed selectivity against *S. epidermidis* ATCC12228 (MIC = 12.5 µg/ml) and *Bacillus subtilis* ATCC 6633 (MIC = 25 µg/ml) strains. Derivatives **1** and **4** showed activity against the same microorganisms but with a much weaker effect – 50 µg/ml and 200 µg/ml for *S. epidermidis* ATCC12228 and *Bacillus subtilis* ATCC 6633 respectively. No bacteriostatic activity was found against the remaining bacteria. An MTT assay was also conducted for derivative **6**, which showed no cytotoxicity for a concentration of 100 µM [20].

Table 1. The results of the study by Finlayson Rhys et al.

	IC50 [µM]				
	T. b. rhod	*T. cruzi*	*L. don.*	*P. falc.* K1	L6
Didemnidine A	59	130	>180	41	24
Didemnidine B	44	82	>160	15	25
Precursor of Didemnidine A	34	88	>190	32	73
Precursor of Didemnidine B	9.9	28	>160	8.4	25
Melarsoprol	0.01				
Benznidazole		1.35			
Miltefosine			0.52		
Chloroquine				0.2	
Podophyllotoxin					0.01

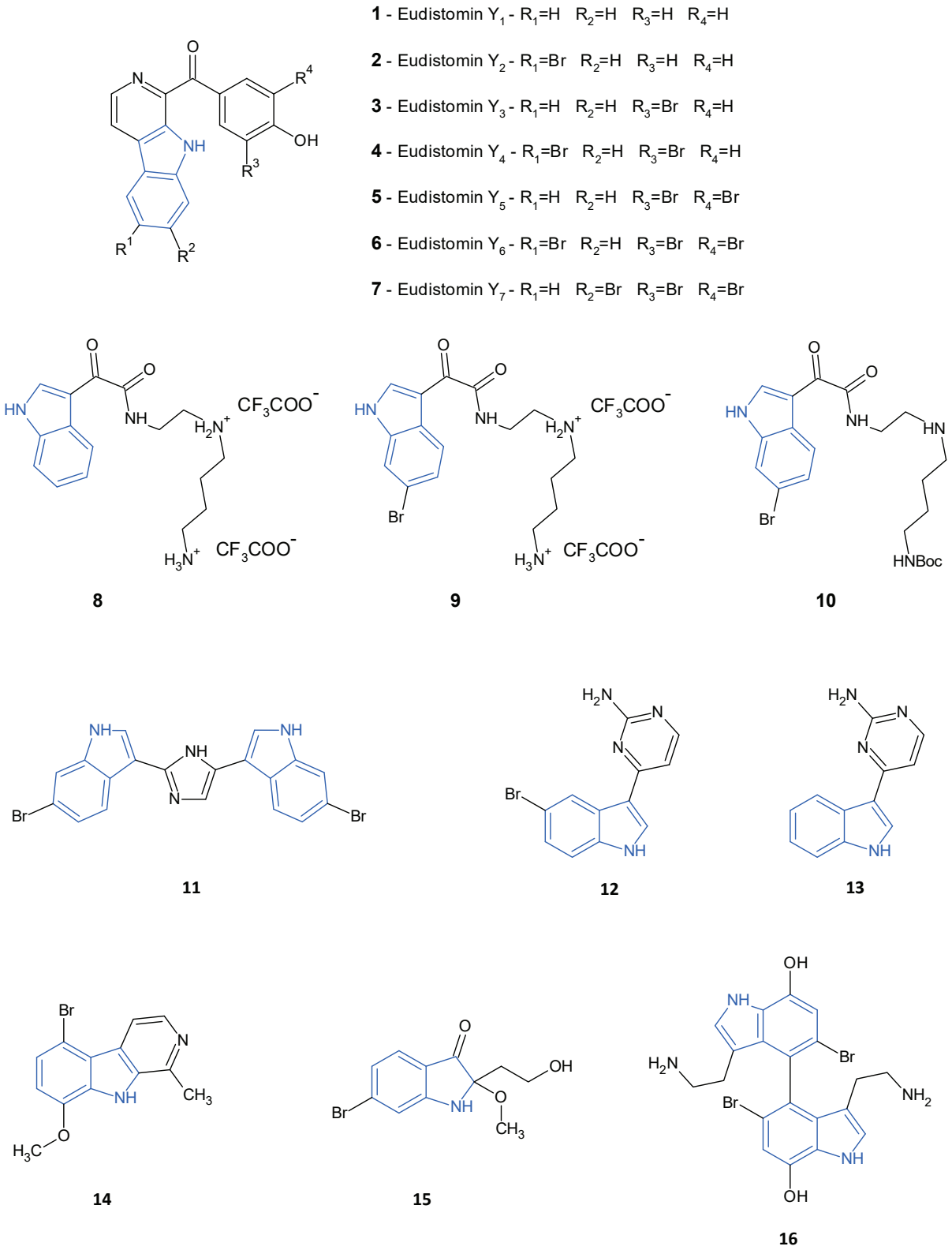


Figure 2. Structures of compounds 1–16 (1–7 – Eudistomin Y1–7; 8 – Didemnidine A; 9 – Didemnidine B; 10 – precursor of 9;11 – nortopsentin A; 12 – Meridianin C; 13 – Meridianin G; 14 – 5-bromo-8-methoxy-1-methyl-β-carboline; 15 – matemone; 16 – Dendridine A).

In their work, Finlayson Rhys et al. isolated certain metabolites of various types of sea squirts from the *Didemnidae* family. After extracting and confirming the structure of these compounds, they synthesized them and described two new derivatives, Didemnidine A (**8**) and Didemnidine B (**9**) (**Figure 2**). The obtained compounds and their precursors were then tested to determine the IC_{50} against selected parasites:

- *Trypanosoma brucei rhodesience*, STIB 900 strain (trypomastigotes stage);
- *Trypanosoma cruzi*, Tulahuen C4 strain (amastigotes stage);
- *Leishmania donovani*, MHOM-ET-67/L82 strain (amastigote/axenic stage);
- *Plasmodium falciparum*, K1 strain (IEF stage).

Drugs from the pharmaceutical market used to treat diseases caused by the above-mentioned organisms were used as controls. Cytotoxicity was determined against the L6 rat skeletal myoblast cell line. Noteworthy is the compound Didemnidine B and its precursor (**10**). With relatively low cytotoxicity, it shows medium growth inhibitory properties against *P. falciparum*. While its precursor is characterized by almost 2 times higher activity. The *T. brucei rhodesienc.* strain was also found to be susceptible to this compound. Although these therapeutic activities are not good enough, the authors' work may serve as inspiration for the development of new series of drugs, e.g. for malaria, which are modifications of Didemnidine B [21].

Two other studies have also described brominated indole derivatives that could be initial compounds for a new antimalarial drug. Alvarado Stephenie et al. tested nortopsentin A (**11**) (**Figure 2**), a compound derived from the sponge species *Spongosorites*, on chloroquine-resistant strains of *Plasmodium falciparum*. This derivative appears promising, as it shows an IC_{50} against this strain of 0.46 µM with a good selectivity index of 14.3 (against the NIH 3T3 fibroblast line). Bharate Sandip et al. in turn described compounds called Meridianins derived from the tunicates of the *Aplidium meridianum* species. Of the entire series, Meridianin C (**12**) and G (**13**) are the most promising (**Figure 2**), showing IC_{50} levels of 4.4–14.4 µM against other *P. falciparum* strains with selectivity coefficients of 25.1 and 24.1, respectively. Additionally, Meridianin C shows moderate activity against *Leishmania donovani* promastigotes (IC_{50} = 64 µM) [22,23].

Another example of potential pharmaceuticals is a group of compounds called β-carboline alkaloids. They have been isolated from animals in the family *Catenicellidae*. This family includes a species of invertebrate animals from New Zealand, *Pterocella Vesiculosa*, which was the subject of research by Till Marisa and Prinsep Michèle. In their work, the researchers isolated and described 5-bromo-8-methoxy-1-methyl-β-carboline (**14**) (**Figure 2**) and tested its inhibitory activity against the Gram-positive bacteria *Bacillus subtilis* (2–4 µg/ml), the fungi *Candida albicans* (4–5 µg/ml) and *Trichophyton mentagrophytes* (4–5 µg/ml) [24].

A compound that could serve as another initial substance for the development of new chemotherapeutics, called matemone (**15**), has been isolated from *Iotrochota purpurea*, a sponge that inhabits the Indian Ocean (**Figure 2**). The compound belongs to the oxindole group and was isolated by Carletti Isabelle et al. The authors tested the antimicrobial activity against *Staphylococcus aureus* by determining the zone of growth inhibition for discs containing 50, 100 and 200 µg/disc which were 7, 9 and 11 mm, respectively. Although the derivative showed bacteriostatic activity, it was not active against *Candida albicans* (at 200 µg/disc) [25].

Another species of sponges from which indole derivatives have been extracted is *Dictyodendrilla*, which resides in Okinawan waters. Tsuda Masashi et al. described the antimicrobial activity of a symmetrical dimer called Dendridine A (**16**) (**Figure 2**). According to the researchers, the compound shows promising results against both the bacteria *Bacillus subtilis* (MIC = 8.3 µg/ml) and *Micrococcus luteus* (MIC = 4.2 µg/ml), as well as the fungus *Cryptococcus neoformans* (MIC = 8.3 µg/ml) [26].

In the context of antibiotic resistance, an important discovery is the work of Zoraghi Roy et al. The researchers were interested in a series of alkaloids from the sponge species *Topsentia pachastrelloides*. The substances cis-3,4-dihydrohamacanthin B (**17**) and bromodeoxytopsentin (**18**) showed the best results (**Figure 3**). Against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, the MIC values for these

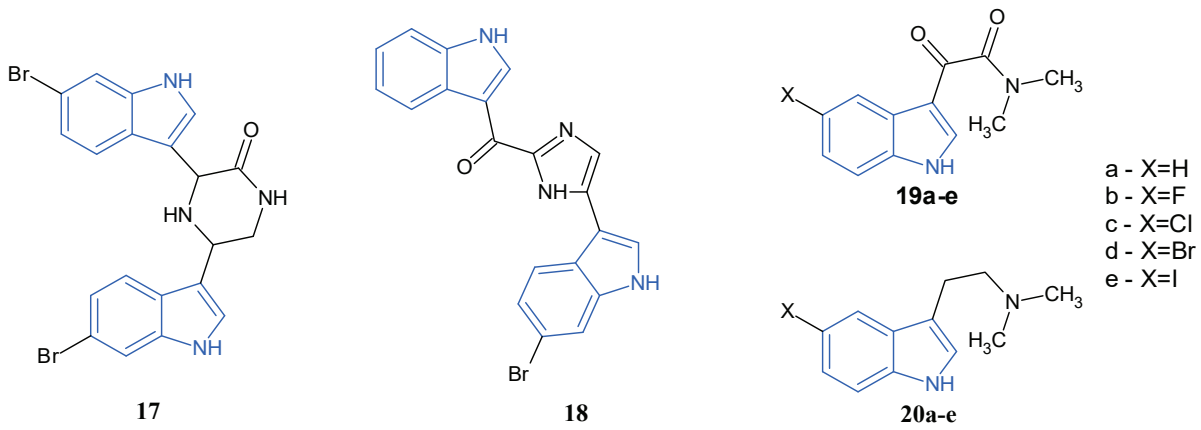


Figure 3. Structures of compounds 17–20a-e (17 – cis-3,4-dihydrohamacanthin B; 18 – bromodeoxytopsentin;19a-e – amide-derivatives of indole; 20a-e – amino-derivatives of indole).

compounds were 12.5 μg/ml and 6.25 μg/ml, respectively [27].

Moreover, brominated indole derivatives have applications beyond antimicrobial therapy. For example, for the above-mentioned matemone and 5-bromo-8-methoxy-1-methyl-β-carboline, anticancer activity has been proven in the same studies. The first one shows moderate anti-cancer activity against NSCLC-N6 L16 lung cancer cells (30 μg/ml), Mia PaCa-2 pancreatic cancer (24 μg/ml) and DU145 prostate cancer (27 μg/ml). The second one, on the other hand, has a relatively good IC_{50} = 5,089 μg/ml against the P388 murine leukemia cell line [24,25]. In addition, Mohamed A. Ibrahim et al. examined a series of halogenated amide- and amino-derivatives of indole (**19a-e** and **20a-e**) (**Figure 3**). In *in vitro* studies, compounds **20a**, **20c**, **20d**, **20e** were shown to have affinity for 5-HT1A and 5-HT7 receptors in the nanomole concentration range. Significant antidepressant-like effects for compounds **19a**, **20d**, **20a**, **20c**, **20e** were proven on mice *in vivo* (forced swim and locomotor activity tests). The authors explain such an increase in affinity for molecular targets in part by the presence of halogen bonding, which was observed during docking to the corresponding receptors [28].

Brominated pharmaceuticals that are approved

Bromine is not only occurring in experimental medicinal substances. Some drugs that have been tested and approved for pharmacological use also contain this element in their structure. Compared to other halogens, such as chlorine or fluorine, bromine is not as widely found. However, the range of pharmacological groups is quite wide. We can find drugs with this element in the following medications (**Figure 4**):

- Anesthetic drugs. The representative of this group is halothane (**21**). This drug was approved in 1956 for usage in general anesthesia. Halothane belongs to the group of inhaled pharmaceuticals, which also includes isoflurane and enflurane.
- Antihistamines. This group includes brompheniramine (**22**), dexbrompheniramine (**23**) and bromodiphenhydramine (**24**), which were registered in 1955s, 1970s and 1980s, respectively. These drugs belong to the first generation of antihistamines and in their days were used quite often alone or in combination with other drugs to treat allergy symptoms and the common cold. Dexbrompheniramine is the active enantiomer of brompheniramine, while bromodiphenhydramine has an additional ether group in its structure.
- Anticancer drugs. In this group we can note two medicines. Pipobroman (**25**) has an alkylating activity due to its two β-bromoamide moieties. Vandetanib (**26**), on the other hand, acts as a kinase inhibitor at various receptors, including VEGFR and EGFR. This pharmaceutical is used to treat thyroid cancer.
- Drugs used in CNS diseases. Necergoline (**27**) is an α_{1A}-adrenergic receptor antagonist and has found application in the treatment of CNS diseases of vascular origin, such as demen-

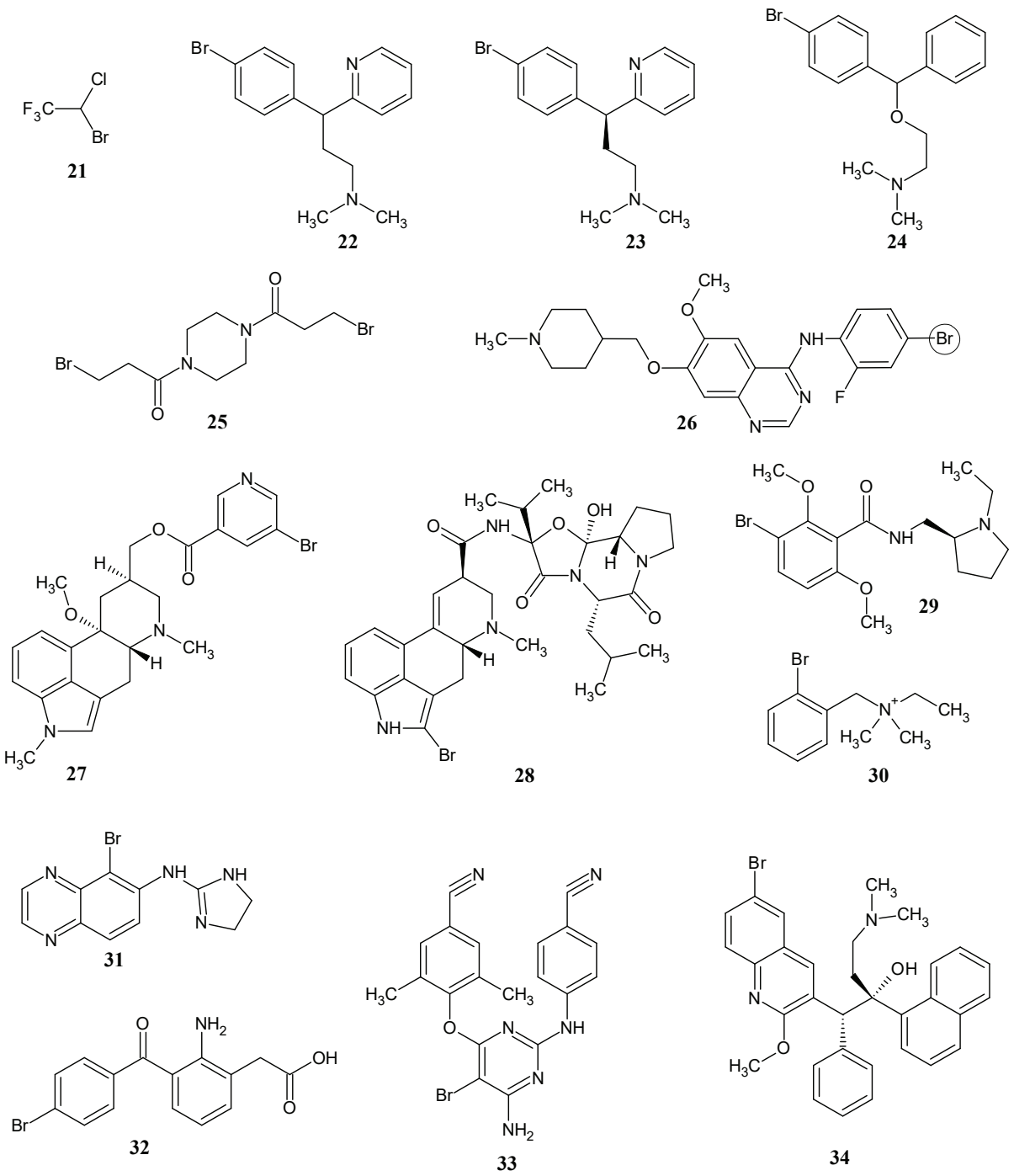


Figure 4. Approved drugs with bromine in their structure.

tia and migraine. Bromocriptine (**28**) is a dopaminergic derivative of ergoline and is used in the treatment of Parkinson's disease. Remoxipride (**29**) although it had success in treating drug-resistant schizophrenia, it was withdrawn quite quickly due to side effects.

› Cardiological drugs. This group includes bretylium (**30**), which was registered in 1959 for the treatment of hypertension. After time, however, it began to be used as an antiarrhythmic drug to treat tachycardia and fibrillation in emergency medicine.

› Ophthalmic drugs. Brimonidine (**31**) as an α-adrenergic medicine has found application in the treatment of open-angle glaucoma since 1996. Bromfenac (**32**) belongs to the NSAIDs and is a selective COX-2 antagonist. It is used to treat inflammation after ophthalmic

surgery since 2000. This drug has good pharmacokinetics and few side effects.

› Biocide drugs. In this category we can include two drugs, which are used in drug-resistant infections. Etravirine (**33**) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and there are no cases of resistance to this medication compared to other drugs in this group. It was approved in 2008 for the treatment of HIV. Bedaquiline (**34**) was registered in 2012 for the treatment of multi-drug-resistant tuberculosis in combination with other drugs.

Bromine in radiopharmacy

Production and labelling methods for bromine radionuclides

In addition to classical therapy, bromine and its isotopes have found applications in radiopharmacy. Radiopharmaceuticals are used in nuclear medicine as diagnostic tools and in therapy. The introduction of radionuclides allows for monitoring the cellular uptake of an active compound *in vivo*. Secondly, modification enables observing biochemical and pharmacological processes at the molecular level. Thirdly the radiation emitted by the radiopharmaceutical can kill and inhibit the proliferation of cancer cells. For mentioned applications, radiohalogens have potential use in this field. The leading halogen for imaging is the β^+ emitter – ^{18}F, used in positron emission tomography (PET). However, the isotope has a short half-life of 110 minutes, preventing monitoring metabolic pathways at later time points[29,30]. Iodine has a broader application range, presented by several isotopes with different decay characteristics. Nevertheless, the binding strength of iodine radionuclides to organic molecules sometimes does not allow the molecule to remain stable in the organism. On the contrary, bromines form stronger bonds in molecules, which increase resistance to unfavorable conditions in the body. Besides their purpose as imaging agents for PET with a longer half-life (^{76}Br), they are applicable as targeted therapeutic agents in treating tumors (^{77}Br, ^{80m}Br – Auger electron emitters. Considering these properties and new methods for producing isotopes and labeling molecules, bromine has potential in a broad spectrum of radiopharmacy [29,31]. Among well-known methods, the isotopes ^{75}Br and ^{76}Br manufacture proceeds through (p, 2n) nuclear reactions with selenium-76 and selenium-77 nuclides. Nevertheless, contamination occurs during the approach due to the competitive (p,n) reaction. Moreover, (p, α) ^{3}He and deuterons-induced reactions on $^{77,78,80}Se$ did not yield satisfactory results either [30]. The high vapor pressure and low boiling point of selenium result in the easy degradation of these isotopes, presenting another drawback. Degradation issues also arise with an α beam bombardment to arsenic. A newly proposed alternative for obtaining ^{76}Br involves heavy-ion fusion evaporation reactions. In the reaction, ^{28}Si transfers energy to natural chromium or ^{16}O to natural copper. McGuinness SR et al. successfully produce ^{76}Br and ^{77}Br isotopes using this method with advantages over the mentioned pathways. Radioisotopic impurities in the technique can be easily isolated using differential cold therapy or dry distillation. Equally significant aspects are characteristic of specific isotopic yield and renewable targets made from copper. High beam currents can also lead to better reaction yields [32]. An innovative approach for obtaining β^- -emitters with a half-life between 1 and 10 days is their extraction from molten salt reactors. For bromine radionuclides, the method applies to isotope ^{82}Br. The described manufacturing route for ^{82}Br is likely cheaper than other procedures. However, the production efficiency of the isotope in the experiment was below 0.02% [33].

Using a radioisotope itself does not enable it to image atypical cells or targeted therapy. The more precise action of radiation-emitting atoms is with a linked compound binding to specific cell receptors. The most commonly used reactions are electrophilic destannylation of the tributyltin precursor. This type of substitution without a carrier often results in lower efficiency due to the high activity of intermediates. Zhou Dong. et al. proposed a similar manufacturing process using a diaryliodonium salt precursor. The approach with microwave irradiation achieved high yields for 4-bromobenzoate and 4-bromobenzaldehyde. Furthermore, selecting a base-free method with subsequent HPLC purification treatment led to low contamination by other products. The particular two compounds are potential precursors for synthesizing radiopharmaceuticals, such as a radiolabeled poly-ADP-ribose polymerase-1 (PARP-1) in cancer imaging [34]. For the radiobro-

mination of specific membrane antigens of cancer cells, the copper-mediated reaction through an arylboronic precursor demonstrated satisfactory efficiency. At room temperature, selecting an appropriate boronic precursor and protecting its carboxyl groups with tert-butyl (t-Bu) groups achieved high labeling efficiency with ^{77}Br at 93%. T-Bu protection avoids the reduction in radiochemical conversion caused by the reaction with a carboxyl group. Other examples of reactions require oxidizing agents or organotin precursors. At a disadvantage, both reactions necessitate thorough product purification [35]. If a peptide or protein is brominated, a pre-conjugation using N-succinimidyl-2,6-dimethoxybenzoate allows the incorporation of bromine into the structure of any peptide with an α or ε-amino group. The presented solution enables a broader application than direct tyrosine residue labeling. Additionally, the presented approach's milder reaction conditions better preserve the peptide's biological activity [36].

Application of radiopharmaceuticals with ^{76}Br and ^{77}Br

Considering the similar properties of bromine and iodine, bromine could be a substitute radionuclide in nuclear medicine. Hashimoto T. et al. compared the isotope ^{77}Br with ^{125}I in a compound based on an inhibitor of p38α, which suppresses the inflammatory response. Bromine has a lower atomic number than iodine and probably will generate more Auger electrons than X-ray emission. In other words, it results in a more substantial destabilizing effect on cancer cells. Regrettably, in both cases, the inhibitory potential was similar. The radioactivity in the blood from the bromine nuclide persisted for at least 2 hours, indicating the stability of the compound. On the contrary to ^{77}Br, accumulation in the inflamed tissue was lower than with ^{125}I [37]. The magnified lipophilicity was the probable reason for low accumulation in the target tissue. In such cases, rapid uptake in the liver and transfer of the compound to the small intestine may occur. In this way, the result is minimal accumulation in the target tissue. Ogawa K. et al. improve the radiopharmaceutical biodistribution by introducing ethylene glycol into the molecule [38].

Another combination for studying the potential use of bromine-77 as a radiotherapeutic is with PARP-1 inhibitors. Incorporating ^{77}Br with rucaparib produced a more potent cytotoxic effect on prostate cancer cells. The Auger electron's high linear energy transfer is lethal but within a short range (10–100 nm). The nuclear localization of PARP molecules and their ability to bind DNA results in a beneficial response. The proximity of ^{77}Br nuclides to DNA significantly enhanced the destructive action in the tumor. Applying hybrid connection and radiation exposure caused cell cycle arrest at the G2-M phase by inducing double-strand DNA breaks. Even a single dose of the compound improved the survival of mice with prostate cancer [39]. *In vivo* studies on mice with ovarian cancer also observed favorable results. The ^{77}Br-rucaparib derivative (**35**) demonstrated specific cellular uptake and a thousand times lower median effective concentration than rucaparib alone. Compared to standalone PARP inhibitors, the hybrid compound showed higher cytotoxicity, independent of BRCA1 expression. Labeling with ^{76}Br in the same experiment did not show such good results. Significant uptake in the liver was observed after administration, followed by accumulation in the gallbladder in subsequent hours [40].

Tyrosine kinase inhibitors are an alternative class of targeting agents in cancer therapy with bromine radionuclides. These molecules can bind intracellularly to cancer cell lines with overexpressed mutated epidermal growth factor receptors (EGFR). Drugs used as tyrosine kinase inhibitors for cancer often do not effectively combat abnormal cells. During treatment, there is an increase in resistance mutations, including those occurring in EGFR. As a consequence, receptor variations create a problem in choosing a drug. On the other hand, immunohistochemical methods for determining EGFR mutations in tumors require tissue biopsies and have low specificity [41]. Radiobromine with rociletinib and osmitinib – drugs targeted at cells with the resistance mutation L858R/T790M of the EGFR receptor has the potential for usage. However, hybrid particles need improvements as therapeutic agents. Radiohalogen with rociletinib (**36**) was specific for double mutations L858R/T790M compared to wild-type EGFR and single mutation L858R. Unfortunately, the complex exhibited high hydrophobicity, which prevented effective tumor accumulation, and the marker lacked stability *in*

vivo. According to authors, there was undesirable accumulation in healthy lung cells, and the tested anticancer efficacy of the ^{77}Br complex was comparable to the non-halogenated compound [42,43].

A promising solution for using ^{77}Br radiotherapeutics in cancer treatment is the application of convection-enhanced delivery based on pressure-driven fluid flow. The procedure could prevent glioma cell growth recurrence after surgical resection. Raghavan R. et al. tested the effect of Auger electron emitters on a surgical resection model of a 2 cm tumor. By calculating the delivered radiation dose from radionuclides to cells, the toxicity to healthy cells would be very low for ^{77}Br. In the least promising calculations, it would be 0.51 Gy for healthy cells and 52 Gy for cancer cells. Most isotopes, including ^{123}I, ^{125}I, ^{99m}Tc, ^{195m}Pt, or ^{193m}Pt, would show higher toxicity to healthy cells [44].

The isotope ^{77}Br is frequently used in cancer research due to its relatively long half-life and dual capability as both an imaging agent (γ-emitter) and a targeted radiopharmaceutical. Instead, the positron-emitting isotopes ^{76}Br and ^{75}Br can have applications for PET imaging. However, ^{75}Br has a significantly shorter half-life than ^{76}Br (1.61 h vs. 16.2 h). Therefore, trials regarding ^{76}Br are more common [30,31]. For metabolic PET imaging of mouse DBT glioma cells, Bartels J.L. et al. tested the ^{76}Br complex with an amino acid. The (S)-amino-2-methyl-4-[^{76}Br]bromo-3-(E)-butenoic acid (**37**) undergoes mixed transport into the brain using amino acid transporters of systems L and A. The same compound, labeled with radionuclide ^{123}I, is transported by system A transporters. Effective transport to the brain via system A route is possible only with a damaged blood-brain barrier. Therefore, the described type of transport is very limited in terms of delivering imaging agents. System A transporters carry neutral and smaller amino acids, while system L transporters carry neutral and large ones. Despite mechanisms of transport, the brominated radiopharmaceutical was the one transported to a greater extent by the system L route. The results indicate that, in this case, the greater electronegativity of bromine and its impact on the molecule's charge were more significant than the larger substituent diameter of iodine. Differences in the compound transport routes resulted in higher uptake in subcutaneous glioma tumors of radiobrominated amino acids [30].

Combining ^{76}Br with meta-bromobenzylguanidine (**38**) presented promising out-turn in vitro for imaging carcinoma tissue expressing norepinephrine transporters. Imaging molecules allowed for detecting even small tumors in the PC-12 cell line. On the other hand, there was significant accumulation in the liver, intestines, stomach, and around the throat 3 hours after injection. Then, the radiopharmaceutical was washed out from non-targeted tissues [45]. The differences in biodistribution can appear when the site of atomic substitution changes. The bromine-76 atom's isomeric position in the aromatic structure influences the effectiveness of radionuclide delivery to the target site. Lang L. et al. demonstrated the relationship using the discussed isotope for potential corticotropin-releasing hormone receptor type 1 (CRF1) imaging. The [8-(4-bromo-2-,6-dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-a][1,3,5]triazin-4-yl]-N,N-bis-(2-methoxyethyl) amine (**39**) exhibited approximately 70 times greater affinity for the receptor than the 3-bromo isomer. Additionally, introducing methoxy groups into the phenolic ring reduced the compound's lipophilicity and subsequent adverse uptake by the liver. The modification resulted in better brain cellular uptake and reduced non-specific binding affinity [46]. Subsequent animal tests also indicated better properties of the 4-bromo derivative. The isomer had a higher affinity for CRF1 binding sites in monkeys' prefrontal cortex than the 3-bromo derivative and the corticotropin-releasing hormone. A missing element of the study is the analysis of *in vivo* biodistribution. The binding evaluation of the compound was conducted only through *ex vivo* autoradiographic studies on the brain [47].

Another evaluated combination of discussed radionuclide is with peptides containing sequence arginine-glycine-aspartic acid (RGD). Lang L et al. studied labeled RGD peptides to determine the expression of the integrin αvβ3 receptor in cancer cells. In a study on mice with glioma xenografts, the combination showed high accumulation in the tumor and comparable binding to the fluorine analog. However, the kidneys – excretory organs for integrin-binding peptides like RGD, also presented significant uptake for the studied molecule [36].

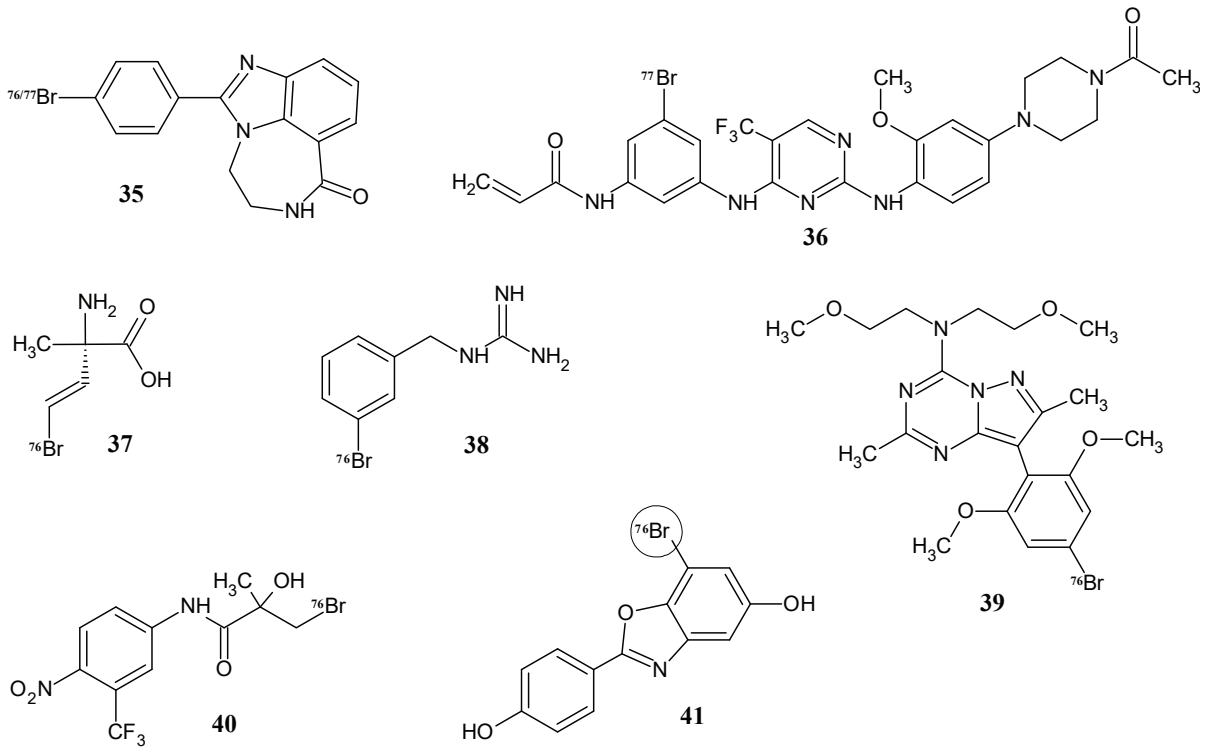


Figure 5 Radiofarmaceuticals with ^{76}Br and ^{77}Br.

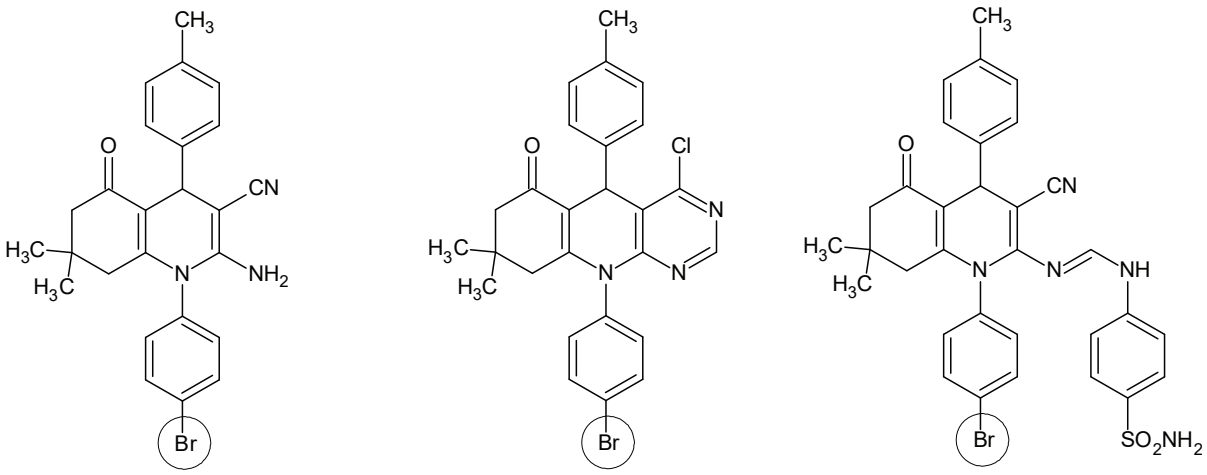
In recent years, proposed ^{76}Br labeled compounds for imaging the occurrence of androgen, adenosine, and estrogen beta (ER-β) receptors were also ineffective [48–50]. The ligand for the androgen receptor, [^{76}Br]-hydroxyflutamide (**40**), had a higher binding affinity than the non-brominated compound. Still, its stability under physiological conditions was shallow, leading to rapid debromination of the complex [48]. Higher binding to the receptor in the halogenated version than the non-halogenated one appeared *in vitro* for the combination with the non-steroidal ER-β ligand (Br-041 (**41**)) – the ERB-041 analog, too. As well as some radiobrominated molecules, it did not have selective uptake by ER-β receptor cells *in vivo*. After administration to the body, there was more significant accumulation in the kidneys and liver than in the target tissue [50]. The same distribution was characteristic of the ^{76}Br – agonist – MRS3581 and the antagonist – MRS5147 of the adenosine receptor [49].

To summarise, studies focus on applying bromine nuclides as PET imaging agents or targeted cancer therapeutic. For the first implementation, research focuses on the radionuclide ^{76}Br. The β^+-emitter has a relatively long half-life, allowing for imaging of metabolic or pharmacokinetic processes at later time points compared to the commonly used ^{18}F. Conversely, for cancer therapy, the Auger electron emitter ^{77}Br is utilized. Its short range of action greatly minimizes damage to healthy cells caused by radiation emission [29–31]. On the other hand, a frequently encountered obstacle with brominated radiopharmaceuticals is their unfavorable biodistribution in tissues after introduction into the bloodstream. One of the reasons is the increased lipophilicity of the compound after introducing the bromine atom, leading to rapid hepatic uptake. Enriching compounds with hydrophilic groups can prevent observed mechanisms [38]. Besides introducing the bromine itself, the position of the halogen substitution also affects the efficiency of compound delivery in the body [46].

Isotopes such as ^{80m}Br, ^{75}Br, and ^{82}Br, despite their therapeutic or imaging purposes, have yet to be studied in recent years. The reason may be the better properties than commonly used radionuclides [31,33].

Influence of bromination on radiosensitization

The manufacture of radiopharmaceuticals requires more production stages than non-la-



42 43 44

Figure 6. Quinoline derivatives with bromine in their structure.

beled chemotherapeutics. For this reason, the effectiveness of introducing natural bromine into the structure of a chemotherapeutic in combination with radiation is also being studied. Presence of bromine in nucleobases with exposition to low-energy electrons caused 2–3 times higher damage to oligonucleotides than in non-halogenated molecules. Their destabilizing potential ameliorates in the following order: 8-bromoadenine > 5-bromocytosine > 8-bromoguanine > 5-bromouracil. However, there are more interactions and bonds in cellular DNA, including hydrogen bonds between the bases of the two strands, which could alter the radiosensitizing effect on the nucleic acid [51]. In turn, Ghorab M. M. et al. studied the synergetic effect of combining a single radiation dose with quinoline derivatives. Of the synthesized compounds, the most active against the MCF7 breast cancer cell line were: 2-Amino-1-(4-bromophenyl)-7,7-dimethyl-5-oxo-4-p-tolyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**42**), 10-(4-Bromophenyl)-4-chloro-8,8-dimethyl-5-p-tolyl-7,8,9,10-tetrahydropyrimido[4,5-b]quinolin-6(5H)-one (**43**), and N'-(1-(4-bromophenyl)-3-cyano-7,7-dimethyl-5-oxo-4-p-tolyl-1,4,5,6,7,8-hexahydroquinolin-2-yl)-N-(4-sulfamoylphenyl)formimidamide (**44**). Each named hybrid molecule showed significantly higher anticancer activity than doxorubicin. After applying a single dose of γ radiation with the presented compounds, the IC_{50} value dropped by about 15 times for compounds **43** and **44** and 5 times for 08. The study results suggest that the mentioned modification can enhance the cytotoxic effect of radiation. Therefore, it is possible to reduce the chemotherapeutic or radiation intensity dose in therapy [52]. Picardi et al. chose porphyrins to study the aftermath of bromination. Enriched porphyrins demonstrated improved radiosensitization and photosensitization compared to their non-brominated versions. It is worth noticing that the authors consider that the strengthened effect is likely due to the higher cellular uptake rather than the enlarged production of cytotoxic species. In the case of photosensitizing, modification led to diminished singlet oxygen release while increasing the mean lethal dose. The same phenomenon could have occurred with enhanced radiosensitization [53].

In turn, modifying a compound with natural bromine can also improve the anticancer effect in radiotherapy. However, the synergistic effect is common to almost all chemotherapeutics. More studies with other molecules will determine the competitiveness of introducing such a modification into anticancer drugs compared to its absence [52].

Halogen bond

To better understand how bromine as a substituent in a potential drug molecule can affect pharmacokinetic and pharmacodynamic parameters, we need to look deeper into the physicochemical phenomena that occur between such a mol-

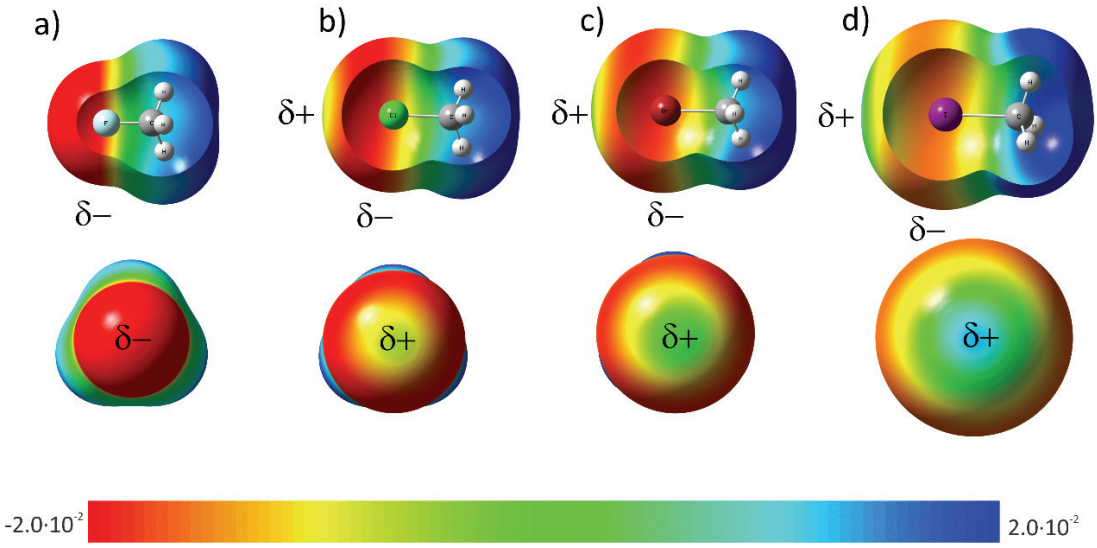


Figure 7. MEP of four halogenometanes: a) fluoromethane, b) chloromethane, c) bromomethane and d) iodomethane. Isosurfaces computed by Density Functional Theory method (functional M06–2X, basis function Def2TZVP).

ecule and a molecular target. The rational design of modern technologies in the fields of chemistry, biology, nanotechnology or medicine takes non-covalent interactions such as halogen bond into account.

Over the years, halogen substituents (-F, -Cl, -Br, -I) in organic molecules have been assumed to be nucleophilic substituents. Indeed, due to their higher electron density, halogens have a partial negative δ^- charge. Thus, they can be hydrogen bond acceptors, among other things. On the other hand, over the past few decades, numerous studies (e.g., molecular electrostatic potential (MEP) mapping, X-ray crystallographic studies) have been performed toward the possibility of halogens forming non-covalent bonds in organic compounds. Thus, the halogen bond (X-bond) was discovered. Through MEP mapping, the charge distribution of halogens in organic molecules was visualized, and it was found that halogen atoms (all except fluorine) do not have a homogeneous negative charge over the entire surface. On the halogen atom, opposite to the R—X bond and on its axis, a deficit in electron density occurs causing a region of positive electrostatic potential called the "sigma hole or σ-hole". Perpendicular to the R-X bond axis zone retains its negative electrostatic potential. The difference in electrostatic potential between the negative (equatorial) and positive (polar) zones of the atom is increasing in the Cl, Br, I order (**Figure 7**).

The σ-hole interacts with Lewis's base or any other electron-rich nucleophilic system to form a halogen bond. Since the observation of the first interactions in which the σ-hole plays a key role, several other types of bonds have been observed such as chalcogen, pnictogen and tetrel bonds [54]. Hence, the acceptor of such an X-bond in organic molecules can be atoms of oxygen, nitrogen, sulfur or the delocalized π orbital of the aromatic ring.

The first historically described compound in which a halogen bond occurs is the adduct of ammonia and the molecular iodine $NH_3 \cdots I_2$, described by M. Colin in 1814. However, a theoretical explanation of this phenomenon had to wait until the 20th century and the development of quantum chemistry. The explanation for this phenomenon remained unclear for a long time until the development of quantum chemistry. The first attempts to explain the formation of the halogen bond was the donor-acceptor model proposed by Mulliken. It involved the transfer of part of the electron density from the orbital occupied by the lone pair electron of the base to the orbitals of the halogen atom[55,56]. A theory describing the mechanism of halogen bond formation based on molecular electrostatic poten-

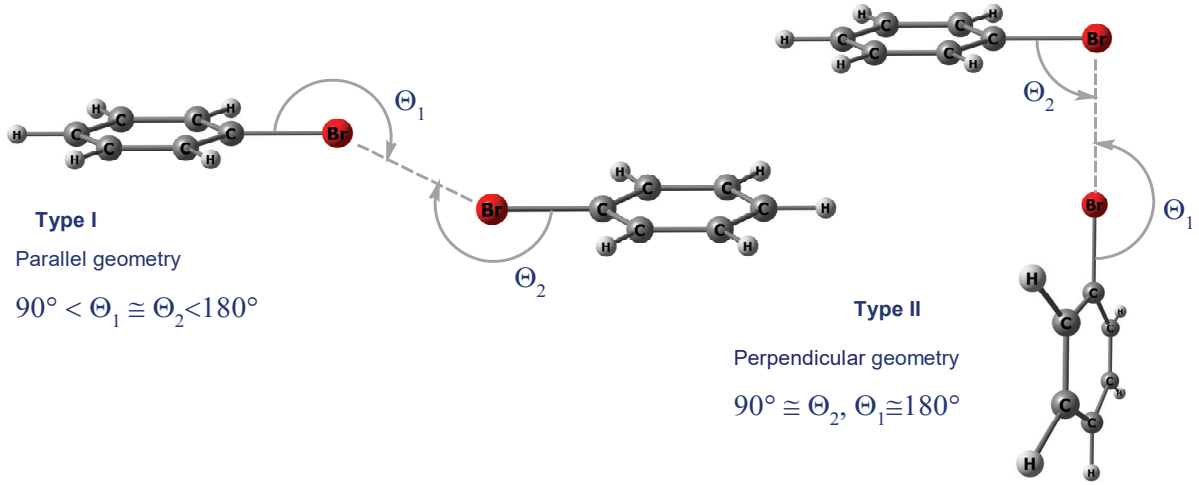


Figure 8. Two possible geometries of halogen-halogen bonds formed in bromobenzenes interactions: type I (parallel) and type II (perpendicular).

tial and sigma hole formation was presented by Polizer and co-workers[56]. Halogen bond energy depends on its type and ranges from 10 kJ/mol (N···Cl) to 150 kJ/mol (I_2···I^-) [57], whereas hydrogen bonds can vary in strength from weak 1 kJ/mol to strong (F—H···F^-) 161.5 kJ/mol [58,59].

According to the definition by IUPAC "a halogen bond occurs when there is evidence of a net attractive interaction between an electrophilic region associated with a halogen atom in a molecular entity and a nucleophilic region in another, or the same, molecular entity" [60]. Expanding on the definition, many data sources, such as protein and nucleic acid databases (PDB), crystallographic structures etc., show the angles between donor and acceptor of halogen bonds, where the angle C—X···Y is close to 180° and the bond length (**d**) X···Y is equal to or less than the sum of the van der Waals radii of these atoms. Consequently, two types of halogen bonds are distinguished – bond with a free electron pair where Y = O, N, S and bond with a delocalized π orbital [61]. In the first case, the angle **α** > 140°. While in the second, **α** > 120° and **θ** < 60° (**Figure 8**, **Figure 9**).

The strength of the halogen bond depends on many factors but mostly it is determined by the size of the σ-hole. Through MEP mapping, Ryan J. et al. discovered a relationship between σ-hole size and the radius of the halogen atom, which increases in the direction Cl < Br < I [62]. On the example of halogenated derivatives of benzodiazepines and their activity against the MDM2 protein, which is responsible for the down-regulation

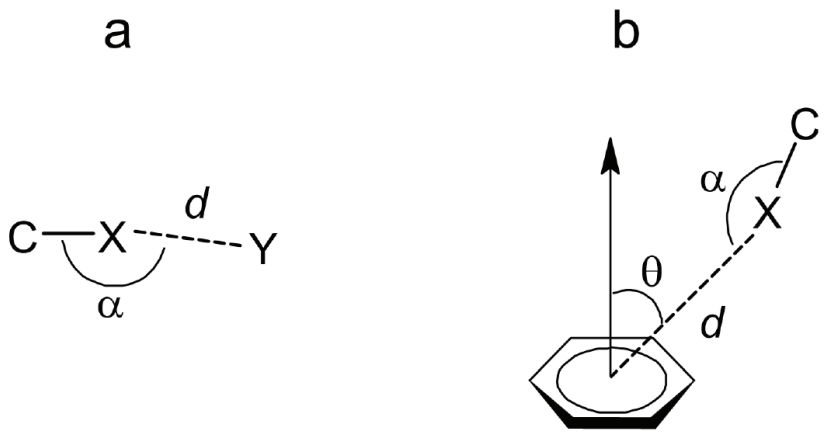


Figure 9. Types of halogen bonds: a – bond with nucleophile, where X = Cl, Br, I and Y = O, N, S; b – bond with aromatic ring.

of the p53 protein, Daniel J. Parks et al. demonstrated a twofold decrease in the affinity of the API for the molecular target after iodine replacement with bromine and a 3.6-fold decrease when substituted with chlorine. This is explained by a decrease in halogen bond strength [63].

The strength of the halogen bond can also be modified by substitution of electron-extracting groups (e.g. -F, -CF_3, -NO_2, -OCH_3, etc.). In their work, Riley K. et al. compared the electrostatic σ-hole potentials of various halobenzene derivatives. Comparing a series of compounds with different configurations of substituents in the ring (chloro-, bromo-, iodobenzene and their difluorinated and pentafluorinated derivatives), the authors concluded that the σ-hole exposure changes directly proportionally with the amount of fluorine in the ring. Namely, the electrostatic potentials of 1-chloro-2,6-difluorobenzene and 1-chloro-3,5-difluorobenzene are comparable to bromobenzene, while 1-bromo-2,6-difluorobenzene and 1-bromo-3,5-difluorobenzene are comparable to iodobenzene. In this way, it is possible to increase the strength of the halogen bond without changing the halogen in the molecule [64]. In a similar study, in addition to calculating the electrostatic potential, calculations were made for cocrystals with 21 X-bond acceptors and it was proven that the more the σ-hole is exposed, the stronger the above mentioned bond [65]. In another of their papers, Aakeröy Christer B. et al. proved the same dependence for aliphatic halo-derivatives [66]. The influence of other electron-withdrawing substituents was also studied by Forni A. et al. In their work, the researchers compared the gas-phase binding energy of formaldehyde with iodobenzene and its derivatives with different substituent configurations [67].

The strength of the halogen bond is also determined by external factors [68]. The medium in which the X-bond complex is formed is important. In the same study, Forni A. et al. compared halogen bond energies in complexes of iodobenzene and formaldehyde derivatives based on calculations in vacuum, diethyl ether and water. It turns out that as the dielectric constant of the solvent increases, the bond X length shortens and the angle of this bond increases. It would be worth expecting an increase in the strength of the bond, but at the same time with this another phenomenon occurs. Namely, the C—I bond gets extended, which plays a significant role in the formation of a halogen bond. Summing up, as the polarity of the solvent increases, the X-bond strength decreases [67].

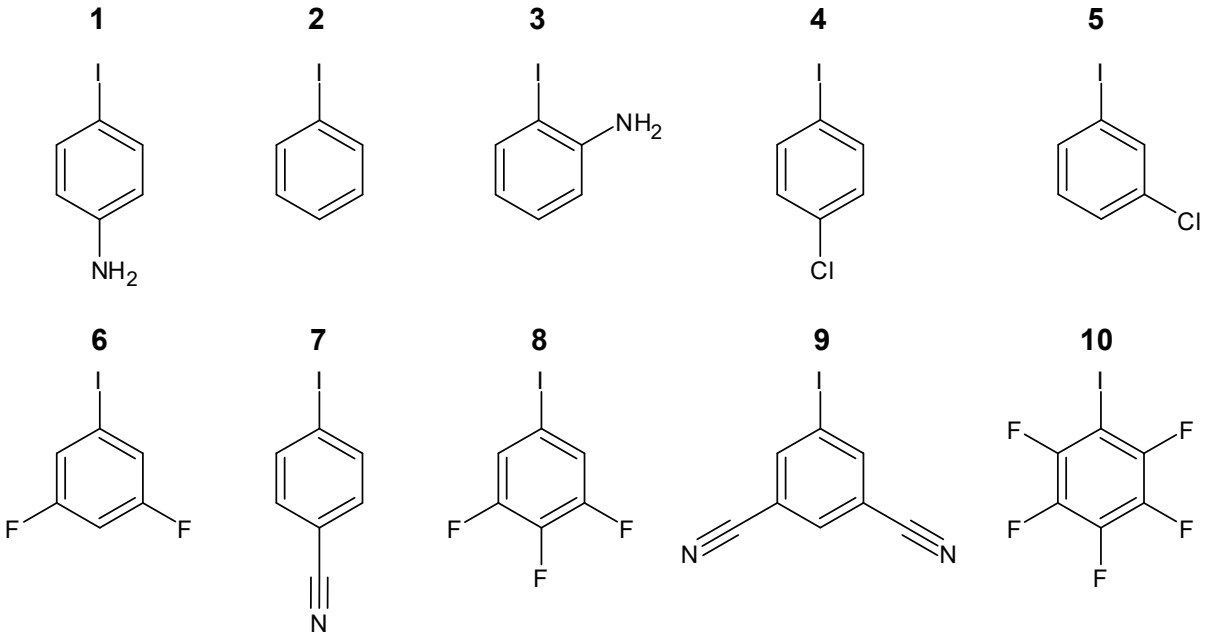


Figure 10. Iodobenzene derivatives for which halogen bond strength was calculated by Forni A [67]. et al. Compounds were placed in order of increase in bond strength (1 – p-aminoiodobenzene, 2 – iodobenzene, 3 – o-aminoiodobenzene, 4 – p-chloroiodobenzene, 5 – m-chloroiodobenzene, 6 – 3,5-difluoroiodobenzene, 7 – p-cyanoiodobenzene, 8 – 3,4,5-trifluoroiodobenzene, 9 – 3,5-dicyanoiodobenzene, 10 – pentafluoroiodobenzene).

So far, halogen substituents in API molecules have been considered as moieties that increase the lipophilicity of compounds. After the discovery of such a phenomenon as halogen bond, scientists began to consider the interactions of halogen-substituted bioactive substances with molecular targets in a slightly different way. It turns out that X-bonding has a significant impact on the interaction of drugs with biomolecules. As mentioned above, halogens form bonds with atoms such as oxygen, nitrogen or sulfur – atoms commonly found in proteins and protein structures of the cell (e.g., enzymes, receptors, etc.). If the molecular target of an API is such a structure, introducing a halogen into its molecule can increase the affinity and strength of binding to that target. At the same time, the X-bond is formed with both the backbone chain of the protein and the side chain [69]. One of the studies confirming the positive effect of halogens on biological activity is the work of Leo A. Hardegger et al. The authors examined a series of modified inhibitors of human cathepsin L (hCatL), an enzyme that, among other things, is responsible for the fusion of viruses into host cells. Through crystallographic analysis, it was proven that halogens form halogen bonds in the S3 pocket of cathepsin which contributes to an increase in the strength of the ligand's binding to the target, resulting in an increase in inhibitory activity. The affinity of such drugs increases 13-fold in some cases. The authors also compared the IC_{50} values of all derivatives. It turns out that chlorine substitution decreases the IC_{50} value by a dozen or even tens of times in some cases. It was also confirmed that substitution of larger halogens contributes to an even greater increase in inhibitory activity [70]. In another study, L. Rohde et al. compared the affinity of five agonists of the α4β2 nicotinic receptor, a therapeutic target for the treatment of certain psychiatric disorders and nicotine addiction. They proved that bromine-substituted APIs have 2–3 times higher affinity for the receptor compared to derivatives without a halogen in their structure [71]. D. Himmel et al. in turn examined the activity of three derivatives, two of which have an iodine atom in their structure, from the group of non-nucleoside reverse transcriptase inhibitors against HIV. The efficacy of these derivatives was compared on a dozen different strains, and it turns out that the halogenated derivatives have better activity against the above mentioned enzyme, making it possible to achieve nanomole or subnanomole IC_{50} values of these APIs. In some cases, the inhibitory effect is enhanced by several hundred times. More importantly, activity was tested against mutants that lack certain binding sites and are resistant to commercially available drugs. As the results show, by introducing a halogen into the molecule, the drug resistance of these mutants can be bypassed because halogen bonds can be formed with other transcriptase binding sites [72]. In addition to binding strength, halogens also affect drug residence time. The X-bonds formed stabilize the complex and significantly reduce the elimination rate of the API from the complex. This allows for the extension of the drug's duration of action, which has a direct impact on its efficacy [73].

Apart from improving the pharmacodynamic parameters of the drug, halogenation also has a positive effect on pharmacokinetic parameters. It is a well-known fact that the more lipophilic a compound is, the better it penetrates biological membranes, which also translates into the bioavailability of the drug. Since halogens in organic molecules were considered substituents that increase lipophilicity, such compounds are expected to have better permeability. This ability, on the other hand, depends on many factors, including the ability to form non-covalent bonds. Speaking in the context of halogen bonds, phospholipids, which are the main building block of biological membranes, have in their structure such acceptors as oxygen from the phosphate residue and carboxylic oxygen derived from fatty acids. In their work, R. Nunes et al. through simulations compared the ability of three benzene derivatives (pentafluoroiodobenzene, iodobenzene and p-iodophenol) to penetrate the phospholipid bilayer and the processes involved in their interaction. The crucial factor turned out to be the halogen bond that was formed during the penetration process. An interesting fact is that X-bonding took place in all stages of membrane penetration and even preceded this process, which allowed the authors to suggest that halogens in the molecule are promoters of passive transport inside the cell which is a property desirable in drug design [74]. Besides, the introduction of halogens into the structure of drugs is a good tool for modifying already existing drugs to improve their activ-

ity. Relatively lipophobic APIs targeting a protein structure have relatively weak properties to bind to the lipophilic part of the peptide (e.g., aromatic amino acids such as tryptophan, phenylalanine) and penetrate this so-called "hydrophobic pocket". Since the π orbital of the aromatic ring can serve as an acceptor for the halogen bond, in this way we can find new binding sites for the API and enhance its action several times or even find other therapeutic applications [75]. In conclusion, halogenation of drugs is a good tool to improve their bioavailability which has a significant impact on therapeutic efficacy.

As it turns out, the presence of halogens in the structure of a molecule also has a significant effect on its metabolism. In the case of prodrugs, their metabolism is a desirable phenomenon. However, it is often the case that the metabolites of a therapeutic substance show much lower activity or no activity at all. In extreme cases, the metabolites can even be toxic. In such cases, when designing a drug, we try to minimize its metabolism or eventually direct it in a different way. Enzymes of the cytochrome p450 family are mainly responsible for metabolism in the body. In itself, the C—X bond is very difficult to break [76]. This can be ascertained by the example of the tert-butyl group.

In their study, Hyeung-geun Park et al. compared a series of TRPV 1 receptor antagonists with analgesic effect. Looking at the two particular examples shown in Fig. 11 which differ only in the methyl group in the tert-butyl substituent, the researchers found similar activity for both derivatives. Prototype **a** was rapidly metabolized, which was associated not only with rapid elimination of the drug from the body, but also its metabolite lost selectivity to the target receptor. Replacing one methyl group with a perfluoromethyl group completely blocked this metabolic pathway at the cost of a slight increase in IC_{50} values, while lowering API clearance by 3.5-fold [77]. In addition to this fact, halogen bonds also affect the metabolism of xenobiotics in our body. In their study, S. Jiang et al. subjected chlorinated benzyl cyanides to the action of nitrilase, an enzyme that is responsible for the conversion of a nitrile group to a carboxyl group. In both cases, the aromatic ring was substituted with only one chlorine atom, while they differed in the position of the fluorine – in the first, the chlorine was in the meta position, in the second – the para. It turns out that the enzyme is selective towards the meta isomer, while it is not active towards the para isomer. It is known that in order for the enzyme to bind to the ligand it is necessary that the substrate is properly located in the active site. The enzymatic reaction occurs only when the substrate-enzyme complex settles into the correct space configuration. In both cases, the researchers observed an X-bond in the substrate-enzyme complex, but the acceptors of this bond were different amino

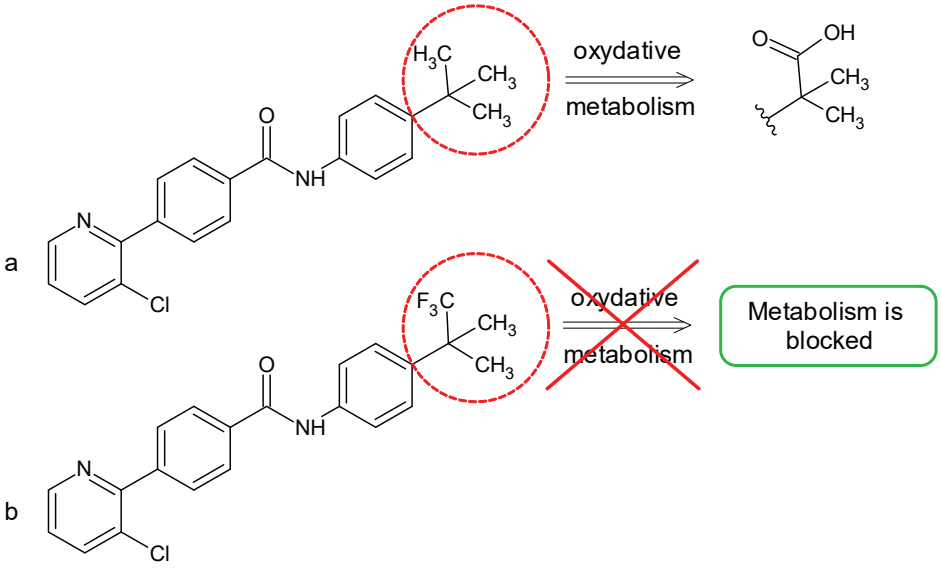


Figure 11. TRPV 1 receptor antagonists with analgesic effect and their metabolism. a – prototype with a tert-butyl group, b – prototype with one methyl group replaced by a perfluoromethyl group.

acids of the nitrilase – for the meta-derivative it was Gly195, while for the para-derivative it was Tyr173. Thanks to this difference, the compounds arranged in a completely different way. The nitrile group for the first derivative was exposed to the enzyme's active site and undergoes a reaction. For the second derivative, the nitrile group was unavailable [78].

Halogen bonds are found not only in xenobiotic-molecular target interactions. These bonds are also present in biomolecules of the human body. A very good example is the group of thyroid hormones (TH). These include thyroxine (T4) and triiodothyronine (T3), which is the more active form of T4. These hormones are very important for the human body, because they are responsible for fat and carbohydrate metabolism, body growth, brain development etc. Numerous studies by analyzing crystallographic structures confirm the presence of halogen bonds in every interaction of TH with other biomolecules of the body. Scientists also believe that halogen bonds play a key role in molecular recognition processes [79–81].

To begin with, it is worth mentioning the transport of THs. As the molecules are strongly lipophilic, certain transporters are required to transport them to their target site. Thyroxine binding globulin (TBG), transthyretin (TTR) and serum albumin (HSA) are responsible for this process in our body. The most important of these is TBG, as it has the highest affinity for T4. This protein has two cavities for binding T4 between helices H and A, and one of the bonds between T4 and TBG is actually the X-bond, which is formed between 3-iodine of thyroxine and oxygen from L269 of this protein. An interesting fact is that for TTR the key affinity factor is the number of iodine atoms in the molecule. T4 has 100% affinity, where T3 and T2 have only fractions of a percent. For this transporter, multiple halogen bonds can be observed at the same time in the hormone-protein complex. HSA z has four T4 binding sites (Tr1, Tr2, Tr3 and Tr4), where in each of them we can observe the formation of an X-bond [79].

In the human body, regulation of TH activity is very important. The iodothyronine deiodinases (Dio) family of enzymes is responsible for this process. These enzymes regulate THs activity by removing iodine atoms from THs molecules and their metabolites in a process called deiodination. The active site of this enzyme contains a peptide with selenium in its structure, which in this case is a halogen bond acceptor. The researchers say that this type of bond is responsible for regioselectivity in the deiodination process. In addition, the researchers hypothesize that the X-bond of I·····Se leads to the polarization of the C—X bond which allows the iodine to cleave from the TH molecule [80,81].

As THs are counted among the hormones that regulate gene expression, there is a family of appropriate nuclear receptors in cells. By analyzing ligand-receptor crystallographic structures, the researchers confirmed the presence of halogen bonds between 3-iodine of T3 and oxygen from F218 of binding pocket [79].

Taking into account the key factor, which is halogen bond, in the interaction between Dio and TH, it is possible to design suitable active substances to regulate the work of this enzyme which would lead to the regulation of TH activity. In several studies and computational research papers, scientists have analyzed the interactions between Dio and polybrominated diphenyl ethers (PBDEs). It turns out that halogen bonds can be observed in the PBDE-Dio complex. In some cases, the affinity of PBDEs is slightly weaker than that of THs, but this studies can serve as inspiration in designing drugs that can be used in diseases associated with TH activity [80].

As seen from the research presented up above, halogen bonds can be a useful tool for drug design. By introducing halogens, for example bromine, into a molecule, we can positively affect the parameters of a potential drug.

Conclusions

The objective of this review article is to explore the role of the bromine atom in drug molecules and its potential use in therapy. To summarize, both positive and negative aspects of it are presented below.

The arguments in favor of bromine are:

- There are natural compounds in nature containing bromine in their structure, which have proven therapeutic activities.
- The above-mentioned molecules can serve as inspiration in the design of new drugs.
- Derivatives from different chemical groups often show bacteriostatic and bactericidal

activity, even against drug-resistant strains. They also often show biocidal activity against other harmful organisms and viruses.

› Due to the occurrence of the halogen bond phenomenon, various parameters of potential drugs (e.g., drug elimination kinetics, drug metabolism, ability to penetrate biological membranes and bioavailability) can be improved compared to analogues without bromine.

› The above-mentioned binding also contributes to effects on drug potency and selectivity toward molecular targets.

› Compounds with various bromine isotopes may find application in radiotherapy. A positive effect has been proven in the treatment of various types of cancer.

› Bromine positively affects the effectiveness of photodynamic therapy as well as radiosensitization (heavy atom effect).

Disadvantages of using bromine in drug design:

› Although bromine contributes to prolonging the duration of action of therapeutics, it can also potentiate cumulative effects in the body, which in turn can lead to toxic effects.

Some bromine compounds including aromatics have proven harmful effects on the human body [82,83].

The presence of bromine in the API molecule and the formation of halogen bonds may not only contribute to improved pharmacokinetic parameters. In some cases, it can increase the affinity of the substance for enzymes, leading to faster metabolism.

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Conflict of interest statement
The authors declare no conflict of interest.

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REVIEW PAPER

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Juvenile Amyotrophic Lateral Sclerosis: a mini review of literature

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ABSTRACT

Juvenile Amyotrophic Lateral Sclerosis (JALS) is a rare type of motor neuron disease that typically manifests before the age of 25. Research findings indicate that the most prevalent gene mutations linked to JALS are *FUS*, *SETX*, *SIGMAR1*, *SPG11* and *ALS2*. In instances of familial occurrence, the gene mutations are predominantly inherited in an autosomal recessive manner, whereas mutations in SETX follow an autosomal dominant inheritance pattern. The clinical manifestations of JALS encompass a combination of upper and lower motor neuron degeneration, and the disease's prognosis can range from rapidly progressive to a more gradual course. Specific gene mutations may give rise to distinct clinical features in addition to the fundamental motor neuron symptoms. Accurate diagnosis of JALS necessitates thorough clinical evaluation and genetic testing, as understanding the hereditary patterns and accompanying characteristics can offer valuable prognostic insights. Timely identification and proper management of JALS are imperative due to its rarity and significant impact on affected individuals.

Introduction

Juvenile Amyotrophic Lateral Sclerosis (JALS) is a term used to describe patients who develop the disease before the age of 25, with symptoms typically appearing in early childhood [1]. This condition shares common characteristics with the adult form, as it involves progressive degeneration of both upper and lower motor neurons. Patients may exhibit a combination of both UMN and LMN signs, reflecting the dual nature of the disease. The presence of both types of dysfunction can complicate the clinical picture but is essential for understanding the progression and impact of the disease on motor function [2,3].

There are several distinctions between JALS and adult ALS. Firstly, 40% of cases of JALS exhibit a discernible genetic origin, in contrast to merely 10% of cases of Adult-Onset Amyotrophic Lateral Sclerosis (AO-ALS) that are attributed to genetic factors [2,4-8]. Secondly, the prognosis and disease progression in JALS vary depending on the gene mutation, ranging from very aggressive to milder, while adult ALS typically follows a uniformly aggressive course, leading to death within 2-3 years. Thirdly, individuals with JALS may present with a syndromic manifestation, affecting other areas of the central or peripheral nervous system in addition to motor neuron degeneration.

The diagnosis of JALS is significantly dependent on genetic analysis and the development of various methods for discovering genetic mutations. JALS is characterized by multiple genetic subtypes, with the most prevalent mutations occurring in the *FUS, SIGMAR1, SPG11, SETX*, and *ALS2* genes [2,9]. The following are descriptions of the JALS fine main subtypes, as also depicted in the table (Table 1) below.

ALS subtype 2

Genetic Details

The *ALS2* gene encodes the protein alsin and is located on chromosome 2q33 [10]. Mutations in *ALS2* gene are linked to an autosomal recessive form of JALS, initially identified in North African populations [11,12] and Middle Eastern populations [13,14].

Clinical Features

In ALS2-JALS, the degeneration of motor neurons begins early and typically advances slowly [15]. An analysis of 21 cases from various sources revealed an average onset age of 4.9 years, ranging from 1 to 20 years [16]. Clinical manifestations which indicate the degeneration of both upper and lower motor neurons, include early emergence of pathological symptoms like spasticity, dysarthria, dysphagia, bladder dysfunction, and sensory abnormalities [17]. Dysarthria may progress to anarthria within the first ten years of life. Some patients with *ALS2* mutations may present with ataxia and dystonia [18]. While cognitive impairment is not a prominent feature, pseudobulbar affect has been noted in several large familial groups [11,16]. Patients with ALS2-JALS may also develop scoliosis during their second decade of life [19]. Apart from JALS, *ALS2* is linked to a range of conditions such as juvenile primary lateral sclerosis [20], childhood-onset hereditary spastic paraplegia [11], and dystonia [21].

ALS subtype 4

Genetic Details

ALS subtype 4 is linked to a mutation in the *SETX* gene. The *SETX* gene, located at genetic position 9q43, encodes senataxin, a DNA/RNA helicase that plays a crucial role in various cellular processes such as DNA repair, replication, recombination, transcription, RNA processing, transcript stability, and translation initiation [22]

Clinical Features

The mutations in the senataxin gene are specifically associated with the autosomal dominant form in JALS and are referred to as ALS4 [23-25]. A study involving 31 patients revealed a gradual progression of the disease, typically starting at the age of 16 [23]. Pathogenic mutations in this gene have also been identified as the cause of Ataxia with Oculomotor Apraxia type 2 (AOA2), a condition characterized by cerebellar ataxia, oculomotor apraxia, neuro-axonal sensorimotor neuropathy, and elevated serum alpha fetal protein levels [26]. The mutations associated with the autosomal dominant form of JALS are typically found within the first 400 amino acids of senataxin, although mutations have been observed throughout the gene, including the helicase domain [27-29].

ALS subtype 5

Genetic Details

ALS subtype 5 is linked to mutations in the *SPG11* gene. This gene encodes the spatacsin protein and is situated in the chromosomal region 15q15-21. Research indicates that both mixed heterozygous and homozygous mutations in the *SPG11* gene are linked not only to the onset of JALS but also to hereditary spastic paraplegia [30,31]. The spatacsin protein is predominantly expressed in neurons of the cerebellar and cerebral cortex. Over 100 pathogenic mutations have been identified, which can result in the absence or dysfunction of the spatacsin protein [32]. Spatacsin plays a role in maintaining cytoskeletal stability and regulating synaptic vesicle transport.

Clinical Features

Mutations in SPG11-JALS usually lead to truncation of the protein. This leads to a functional impairment, which is essential for its involvement in preserving cytoskeletal integrity and modulating the transport of synaptic vesicles [2,31]. The onset of SPG11-JALS typically occurs between 7 to 23 years of age, with the disease lasting around

34.3 years and following an autosomal recessive pattern of inheritance [31]. Bulbar symptoms of SPG11-JALS often manifest early in individuals, while cognitive impairments and mental health issues are less common. Mutations in the spatacsin gene are also associated with hereditary spastic paraplegia (HSP), but the clinical presentation of HSP patients differs from that of SPG11-JALS patients [33]. Magnetic resonance imaging (MRI) of HSP patients typically shows thinning of the corpus callosum, a feature not observed in SPG11-JALS cases [31].

ALS subtype 6

Genetic Details

ALS subtype 6 is caused by genetic mutations in the *FUS* gene. The *FUS* gene, located at position 16p11.2, encodes the fused in sarcoma (FUS) protein which plays a role in RNA processing [34]. This suggests that disruptions in RNA metabolism may be a contributing factor to the development of ALS [35].

Clinical Features

Mutations in the *FUS* gene are predominantly linked to JALS [9,36]. A study analyzing 38 cases of FUS-JALS [1] found that most cases were due to new mutations. Onset of symptoms typically occurs around the age of 21. Patients with FUS-JALS exhibit both upper and lower motor neuron dysfunction, presenting with spasticity and hyperactive tendon reflexes [37]. The disease progression of FUS-JALS is rapid, leading to death from respiratory failure within 1-2 years [38]. Some cases of FUS-ALS have also shown cognitive impairment, and indeed it has been observed frontal lobe atrophy and abnormalities in functional magnetic resonance imaging (fMRI) [39,40].

ALS subtype 16

Genetic Details

ALS subtype 16 is linked to mutations in the *SIGMAR1* gene, which encodes a molecular endoplasmic reticulum (ER) chaperone and is highly expressed in spinal motor neurons. This gene is located on chromosomal region 9q13.3 and is prominently expressed in motor neurons of the brainstem and spinal cord [41]. The *SIGMAR1* gene is involved in various processes such as lipid metabolism, ER stress response, initiation of autophagy, and calcium metabolism, all of which play a role in neurodegeneration. Studies have shown that inactivation of *SIGMAR1* causes mitochondrial dysfunction, dysregulation of calcium hemostasis, and neurodegeneration in cultures of primary motor neurons.

Clinical Features

In terms of clinical presentation, mutations in the transmembrane region [42] and frame-shift mutations upstream at position 95 (p. L95fs)

Table 1. Main subtypes of juvenile ALS and their genotype–phenotype correlations.

Type	Gene	Locus	Protein	Inheritance	Phenotype
ALS2	*ALS2*	2q33.1	alsin	AR	AAO: juvenile; Onset: LL, UL; PLS, IAHSP; Progression: gradual; UMN dominant > UMN + LMN
ALS4	*SETX*	15q15.1	senataxin	AD	AAO: juvenile > adult; Onset: LL > UL; AOA2, cerebellar ataxia, motor neuropathy; Progression: gradual; UMN + LMN > LMN dominant
ALS5	*SPG11*	15q15.1	spataxin	AR	AAO: juvenile > adult; Onset: bulbar, limb; HSP, autonomic dysfunction, intellectual disability; Progression: gradual; UMN dominant > UMN + LMN
ALS6	*FUS*	16p11.2	fused in sarcoma (FUS)	AD, AR, De Novo	AAO: adult > juvenile; Onset: UL, bulbar > LL; PMA, Parkinsonism, essential tremor, intellectual disability; Progression: swift > gradual; UMN + LMN > LMN dominant
ALS16	*SIGMAR1*	9q13.3	chaperone	AR	AAO: juvenile; Onset: LL > UL; motor neuropathy; Progression: N/A; UMN + LMN

Abbreviations: AAO; age at onset, AD; autosomal dominant, ALS; amyotrophic lateral sclerosis, AOA2; ataxia and oculomotor apraxia type 2, AR; autosomal recessive, HSP; hereditary spastic paraplegia, IAHSP; infantile onset ascending hereditary spastic paralysis, LL; lower limb, LMN; lower motor neuron, N/A; not available, PLS; primary lateral sclerosis, PMA; progressive muscular atrophy, UL; upper limb, UMN; upper motor neuron

have been described [43]. Individuals with SIGMAR1-JALS typically experience onset at 1-2 years of age with muscle weakness and spasticity. Especially, the distal muscle weakness initially affects the hands and forearms, eventually progressing to involve the proximal muscles, leading to complete atrophy of the forearm extensors and triceps [42]. However, no bulbar involvement has been reported and cognition is preserved. It is worth noting that SIGMAR1-ALS in adults may be associated with frontotemporal dementia (FTD), although cognitive impairment is not observed in SIGMAR1-JALS [43,44]. Furthermore, *SIGMAR1* mutations have been linked to distant hereditary motor neuropathy (dHMN) [45] and reduced levels of *SIGMAR1* have been found in the spinal cord of ALS patients [46].

Discussion

Diagnosing JALS poses a challenge due to its rarity and similarities with other motor neuron diseases [2]. The clinical presentation of JALS can vary depending on the specific gene mutation involved, with common symptoms including progressive muscle weakness and atrophy, spasticity, hyperreflexia, bulbar symptoms, and cognitive and behavioral changes [2,8].

Electrodiagnostic studies, such as electromyography (EMG) and nerve conduction studies, play a crucial role in confirming the presence of denervation and reinnervation in multiple myotomes, supporting the involvement of lower motor neurons. Findings may include fibrillation potentials, positive sharp waves, increased amplitude and duration of motor unit potentials, or reduced recruitment of motor units [2].

MRI of the brain and spinal cord can reveal non-specific changes or features related to the underlying genetic mutation. Findings may include cortical and spinal cord atrophy, corticospinal tract signal changes, frontal cortical atrophy, thin corpus callosum, and leukoencephalopathy in some cases [8].

Genetic testing is essential for diagnosing JALS and identifying the specific gene mutation responsible. Understanding the genetic cause can provide valuable information for prognosis and management [2,8]. There are further recorded instances of JALS resulting from mutations in genes commonly associated with adult ALS, such as *SOD1* (Copper/zinc superoxide dismutase-1), *UBQLN2* (Ubiquitin-like protein, specifically ubiquilin 2), and *TARDB* (TAR DNA binding protein), which have been thoroughly examined in existing literature. Alternatively, mutations in other genes like *SPTLC1* (Serine palmitoyltransferase, long-chain base subunit 1) [47], *ERLIN1* (Endoplasmic reticulum lipid raft-associated protein 1) [48], *GNE* (glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase) [49], *VRK1* (vaccinia-related kinase 1) [50], *BICD2* (BICD cargo adaptor 2) [51], *SYNE1* (spectrin repeat containing the nuclear envelope protein 1) [52], *DDHD1* (DDHD domain containing 1) [53], and *CLEC4C* (C-type lectin domain family 4 member C) [51,52,54] have also been identified as leading to clinical manifestations resembling JALS, especially if symptoms manifest before the age of 25.

Other clinical conditions that may present similar symptoms to JALS include Juvenile Primary Lateral Sclerosis (JPLS) and HSP, both of which primarily exhibit upper motor neuron lesion signs like spasticity and dysarthria. HSP may have an earlier and more rapid onset of symptoms, while JPLS may be characterized by abnormal oculomotor findings [55]. Mutations in genes such as *alsin*, *SPG11*, and *ERLIN1* have been associated with HSP. Additionally, Spinal Muscular Atrophy (SMA) and distal hereditary motor neuropathy (dHMN), which predominantly affect the lower motor neuron, can also present symptoms similar to JALS. A case study reported a 10-year-old girl with a mitochondrial neurodegenerative disease showing clinical features resembling JALS, but further investigation revealed abnormal iron accumulation in the basal ganglia due to a compound heterozygous mutation in the C19orf12 gene [56]. Mutations can either be passed down from one parent or arise spontaneously in an individual with the disease. The mode of inheritance, whether autosomal recessive or autosomal dominant, depends on the specific gene involved. Nevertheless, the majority of cases of JALS are of the familial form.

The treatment modalities available for JALS are not specifically defined, with patient management primarily aimed at alleviating symptoms and enhancing mobility. Pharmacological interventions are frequently employed to combat fatigue and diminish muscle cramps. The for-

mulation of personalized treatment plans necessitates collaborative efforts among multidisciplinary healthcare teams [57-61]. Significant advancements have been made in the exploration of potential therapeutic strategies. A prominent area of research focuses on the influence of genetic factors in JALS. Investigations have revealed various genetic mutations, including those in the *SOD1* gene, which may play a role in the disease's onset [62]. By elucidating the genetic underpinnings of juvenile ALS, researchers aspire to create targeted therapies that address the specific molecular pathways implicated [62]. Furthermore, there is ongoing research into innovative neuroprotective strategies, such as the modulation of oxidative stress, the reduction of protein aggregation, and the alleviation of inflammatory responses [63]. These approaches aim to decelerate or potentially halt the disease's progression by targeting the fundamental pathogenic mechanisms responsible for motor neuron degeneration. Another promising research direction involves the exploration of stem cell-based therapies for juvenile ALS. These therapies seek to replace or support damaged motor neurons, thereby aiming to restore functionality and enhance the quality of life for affected individuals. As research progresses, the scientific community remains optimistic that the advancement of more effective treatments for juvenile ALS will yield improved outcomes and enhance the prognosis for those impacted by this debilitating condition [62-65].

Conclusion

In conclusion, the diagnosis of JALS necessitates a high level of suspicion, a thorough clinical assessment, electrodiagnostic studies, genetic testing, and neuroimaging. It is important to distinguish JALS from other motor neuron diseases, spinal muscular atrophies, and hereditary spastic paraplegias [8]. A comprehensive clinical evaluation, along with electrodiagnostic studies and genetic testing, is essential for establishing the diagnosis of JALS and ruling out other conditions. Early recognition is crucial for guiding management and providing appropriate genetic counseling to the patient and their family.

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Conflict of interest statement
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Acknowledgements

Under acknowledgements please specify contributors to the article other than the authors accredited. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.). Also acknowledge all sources of support (grants from government agencies, private foundations, etc.). The names of funding organizations should be written in full.

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This result was later contradicted by Smith and Murray [3].
Smith [8] has argued that...
Multiple clinical trials [4–6, 9] show...

Journal names should be abbreviated according to Index Medicus. If available alwaysprovide Digital Object Identifier (DOI) or PubMed Identifier (PMID) for every reference.

Some examples

Standard journal articles

1. Petrova NV, Kashirskaya NY, Vasilyeva TA, Kondratyeva EI, Marakhonov AV, Macek Jr M, Ginter EK, Kutsev SI, Zinchenko RA. Characteristics of the L138ins (p.Leu138dup) mutation in Russian cystic fibrosis patients. JMS [Internet]. 2020 Mar 31;89(1):e383. doi: 10.20883/medical.383.

Books

Personal author(s)

1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

Editor(s) or compiler(s) as authors

2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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