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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

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The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

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ORIGINAL PAPER

JMS Journal of Medical Science

Impact of additional intravenous methylprednisolone pulse therapy on the quality of life in patients with dysthyroid optic neuropathy

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ABSTRACT

Introduction. Dysthyroid optic neuropathy (DON) is a severe complication of Graves' orbitopathy (GO). Treatment of DON should involve immediate administration of intravenous methylprednisolone (ivMP) in very high doses. It is recommended to include additional 12 pulses of ivMP according to a weekly schedule as a further step of the treatment process. The purpose of this study was to evaluate the influence of a 12-week ivMP treatment on the quality of life (QoL) in DON patients.

Material and Methods. A retrospective study was conducted on 6 patients (the tests involved 8 individual eyes) with DON and treated with ivMP in very high doses, followed by orbital decompression in one patient. All patients were qualified for additional treatment with ivMP in a 12-week protocol and completed the Polish version of the GOQoL questionnaire before and after the therapy. Visual acuity (VA) and diplopia were examined prior to the administration of ivMP pulses for DON, as well as before and after the additional ivMP treatment.

Results. A minimal clinically important difference in QoL was observed in four patients at the end of the additional ivMP therapy. A significant increase in VA was observed following additional pulses of ivMP compared to the evaluation at the time of the DON diagnosis (p=0.04).

Conclusions. Applying additional 12 pulses of ivMP following DON therapy may impact QoL. Performing QoL assessment throughout the entire therapy in patients with DON is particularly important in the clinical practice. Final evaluation of QoL should be performed after completing the entire therapeutic process, which involves surgical treatment to correct diplopia.

Introduction

Graves' orbitopathy (GO) is an autoimmune orbital disorder manifested by disfiguring proptosis, diplopia, pain, redness and swelling of the eyelids [1,2]. The pathogenesis is based on inflammation, adipogenesis, and the production of glycosaminoglycans, which may lead to the expansion of the

orbital connective tissue and the enlargement of the eye muscles [3]. Approximately 5% of the GO patients suffer from dysthyroid optic neuropathy (DON). This sight-threatening complication results from optic nerve compression caused by swollen muscles and fat in the orbital apex [4,5]. The first-line treatment of DON recommended by the European Group on Graves' Orbitopathy (EUGOGO) consists of intravenous methylprednisolone (ivMP) pulse therapy (500–1000 mg for 3 consecutive days). In the case of poor or absent response within 2 weeks, urgent orbital decompression should be performed. Furthermore, it is recommended that patients with a complete recovery receive additional treatment with 12 pulses of ivMP scheduled every week [6].

Up to now, there are no data to verify the influence of additional therapy with ivMP in a 12-week protocol on the quality of life (QoL) in DON patients. Nevertheless, it has been found that moderate-to-severe GO negatively affects patients' well-being. Persons with GO suffer from poorer QoL, both with regard to vision problems and compromised appearance, compared to healthy individuals [7,8]. By means of the National Eye Institute Visual Function Questionnaire (NEI VFQ-25) Du Y et al. demonstrated that vision-related QoL tends to be more impaired in GO patients with DON than in those not suffering from DON [9]. However, NEI VFQ-25 is not a specific QoL questionnaire for patients with GO, and it does not concern some of the unique issues affecting patients with GO, such as altered appearance.

According to EUGOGO, the evaluation of QoL should constitute an integral part of management in patients with GO. Therefore, it is recommended to use specific GOQoL questionnaire, which has been proven to be valid, reliable, and culturally applicable[10]. In 2015 a validated Polish version of the GOQoL questionnaire (GOQoLpl) was developed and subsequently published by EUGOGO as the recommended version for the assessment of QoL among Polish patients with GO in the clinical practice [11]. Since the development of the GOQoLpl only a handful of studies evaluating QoL of GO patients have been conducted, and no study has been performed regarding DON.

The purpose of this study was to evaluate the impact of additional ivMP treatment in a 12-week protocol on the quality of life of DON patients.

Material and Methods

Patients

Six individuals diagnosed with DON were retrospectively recruited in the study. A total number of 8 eyes were affected by DON. Patients were treated in the Department of Internal Medicine and Endocrinology, Medical University of Warsaw, between 2015 to 2018. The diagnosis of DON was based on at least two features, such as deterioration of visual acuity (VA) (< 1.0) and/or colour vision, optic disc swelling and/or signs of DON in magnetic resonance imaging (optic nerve stretching and/or presence of apical crowding) [12]. The inclusion criterion was an additional treatment with ivMP in a 12-week protocol following the treatment of DON. The exclusion criteria were: a previous history of ivMP therapy for GO, as well as a lack of a completed GOQoL questionnaire before or after the additional treatment with ivMP.

Treatment

All patients were administered ivMP (3 × 1.0 g given within three consecutive days). Due to the poor improvement one individual received additional pulses of ivMP (3 × 1.0 g and 3 × 0,5 g – cumulative dose of 8 g) and another one underwent additional endoscopic intranasal orbital decompression. Subsequently each patient was qualified for treatment with 12 pulses of ivMP. Five patients received an additional cumulative dose of 4.5 g (0.5 g once weekly for 6 weeks, followed by 0.25 g once weekly for 6 weeks). One patient (treated with 8 g IVMP for DON) was qualified for 12 pulses of ivMP with a cumulative dose of 7.5 g (0.75 g once weekly for 6 weeks, followed by 0.5 g once weekly for 6 weeks), but due to the increased level of alanine and aspartate aminotransferases this patient received 9 pulses of ivMP.

Laboratory and ophthalmic evaluation

The serum levels of thyroid-stimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4) and TSH binding inhibitory immunoglobulin (TBII) were evaluated with an electro-chemiluminescent immunoassay performed on a Cobas 6000 analyser from Roche Diagnostics (Mannheim, Germany).

VA of the patients' eyes diagnosed with DON was verified using Snellen charts and expressed

as a decimal fraction. The Gorman score was used to evaluate and classify diplopia graded from 0 – 3: 0–no diplopia, 1-intermittent diplopia, 2-inconstant diplopia, 3-constant diplopia [13]. Each parameter was assessed at three time points: prior to the administration of the first-line treatment with ivMP pulses for DON, before and after the additional ivMP treatment.

QoL questionnaire

At the beginning of and after the treatment with additional pulses of ivMP, all patients completed GOQoLpl. It consists of two subscales which assess: limitations in visual functioning (7 questions) and influence of the GO on appearance (8 questions). Each question is scored based on a 3-point scale referring to GO impact: 1–serious, 2-little, 3-no impact. The results are established on the basis of the following formula: (total score- #)/(2 × #) × 100 where # indicates the number of completed items. The total QoL score is expressed as a number between 0 to 100, where a higher result indicates better QoL.

According to Wiersinga et al., a minimal clinically important difference (MCID) in the GOQoL for immunosuppression and surgical decompression is a change of ≥ 10 points [14].

Statistical analysis

All the analyses were performed using SPSS statistical software version 22.0 (IBM SPPS Statistics, New York, US). The results were expressed as a mean (± standard deviation) except for the GOQoL score, which was expressed as a median (interquartile range). Categorical variables were expressed as numbers (n) and percentages (%). The Shapiro-Wilk test was applied to confirm or reject the normal distribution of each continuous variable. Comparisons between continuous data were performed using a paired t-test (for parameters with normal distribution), or the Wilcoxon rank sum test (for parameters with the distribution deviations). Statistical significance was established for the results with $p<0.05$.

Results

The demographic details and clinical characteristics are presented in Table 1. DON was diagnosed in 6 patients in the total of 8 eyes (2 patients with bilateral DON and 4 patients with unilateral DON). VA significantly increased following the additional treatment with ivMP compared to the baseline evaluation at the time of the diagnosis of DON

Table 1. Baseline demographic and clinical characteristics at three time points: prior to the administration of ivMP for DON, before and after the additional treatment with ivMP pulses

Demographics			
Age, years[a]	69.33 (±5.79)		
Male/female[b]	2/4		
Number of eyes with DON	8		
	0	**I PULSE**	**LAST PULSE**
Clinical characteristics of thyroid disease			
TSH (0.27–4.2 μIU/mL)[a]	1.14 (±1.42)	0.68 (±0.52)	1.21 (±0.79)
fT3 (3.1–6.8 pmol/L)[a]	4.64 (±1.80)	4.66 (±1.33)	4.33 (±0.94)
fT4 (12–22 pmol/L)[a]	19.06 (±4.28)	15.72 (±2.61)	17.37 (±3.33)
TBII (< 1.75 IU/L)[a]	10.44 (±7.17)	6.24 (±4.12)	3.47 (±2.53)
Clinical characteristics of orbital disease			
Gorman score[b]			
No diplopia	*3*	*1*	2
Intermittent diplopia	*1*	*1*	1
Inconstant diplopia	*1*	*1*	1
Constant diplopia	*1*	*3*	2
Visual acuity[a]	*0.58 (±0.27)*	*0.78 (±0.29)*	0.83 (±0.23)

[a] Data are presented as means (± standard deviation). [b] Data indicate the number of patients
ivMP: intravenous methylprednisolone, DON: dysthyroid optic neuropathy. 0: diagnosis of DON, I PULSE and LAST PULSE: before and after the additional treatment with ivMP pulses respectively

(p=0.04). A significant improvement has not been observed either between the assessment prior to the treatment of DON and the beginning of the additional therapy (p=0.125), or between the 1st and the last pulse of the additional therapy (p=0.18). Mean values of VA are shown in Figure 1.

All six patients completed GOQoLpl. The median GOQoLpl total score, as well as subtotals for visual functioning and appearance before and after the ivMP treatment are provided in Figure 2. MCID was observed in four patients (2 patients – an improvement of QoL, 2 patients – a dete-

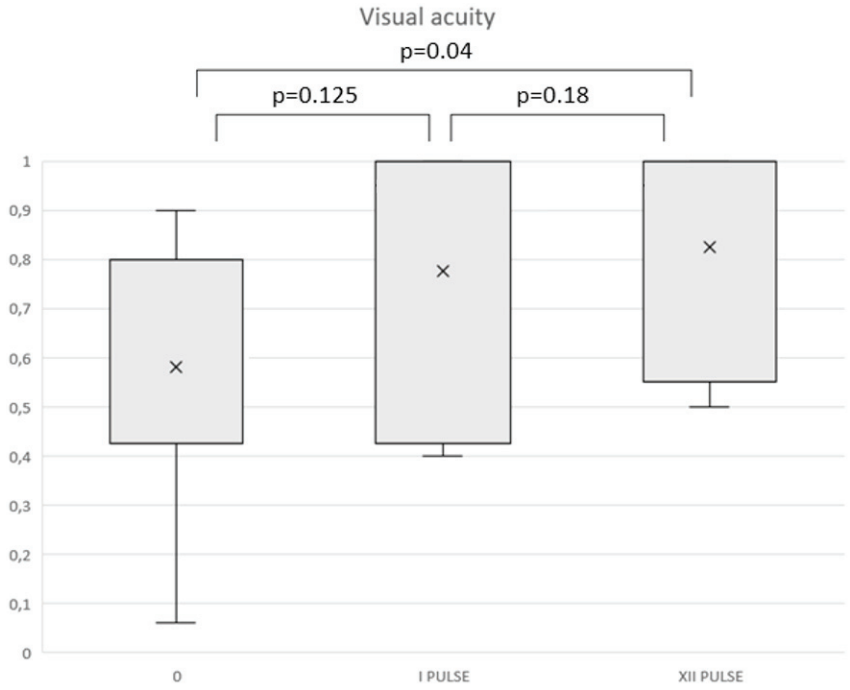

Figure 1. Visual acuity of 8 eyes in patients diagnosed with dysthyroid optic neuropathy (DON). A comparison of variables between the time of the diagnosis of DON (0), the 1ST and the last pulse of additional intravenous methylprednisolone treatment. Vertical line ranges from a maximum to a minimum value. Bold square presents a standard deviation. x – mean

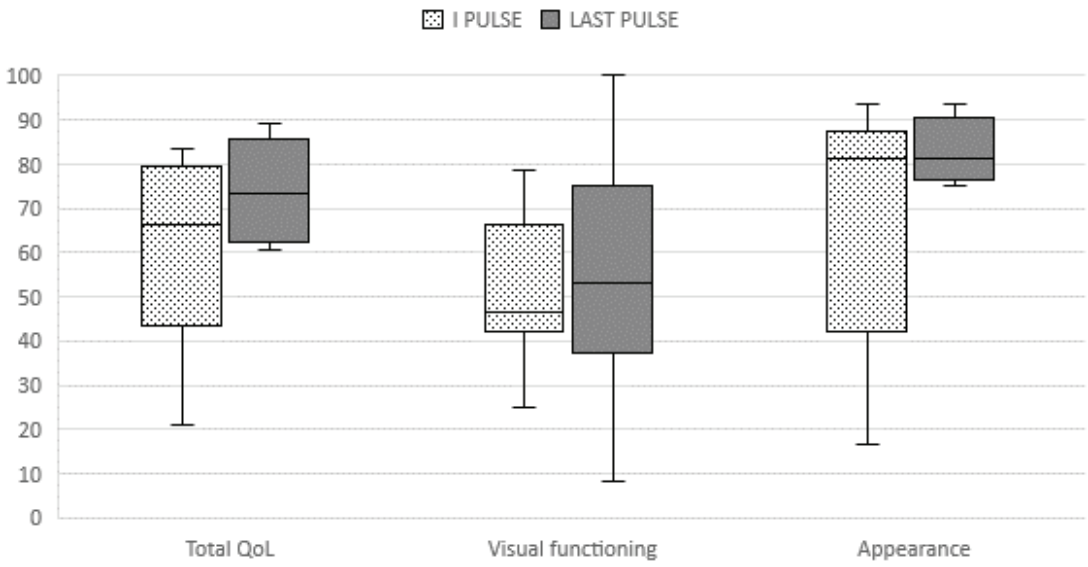

Figure 2. Total Graves' Orbitopathy Quality of Life (QoL) questionnaire score and its subscales: visual functioning and appearance were based on the following formula: (total score- #)/(2 × #) × 100 where # indicates the number of completed items. The total QoL score varies from 0 to 100. Higher result indicates better QoL. A comparison of variables between the 1ST and the last pulse of intravenous methylprednisolone treatment. Vertical line ranges from maximum to minimum value. Data are shown as median values (line across the box) with interquartile (25th – 75th percentile) range (the box)

rioration of QoL). In two patients MCID was not detected.

One of the patients with a deterioration of QoL after the additional treatment, who initially exhibited monocular vision in the course of DON, developed diplopia, due to the improvement of VA during the additional treatment. Furthermore, we found a reduction of eye motility with a deterioration of diplopia in another patient.

Discussion

According to the EUGOGO, the treatment of DON involves immediate administration of ivMP in very high doses followed by urgent orbital decompression as a second-line treatment in the case of poor, or absent response within 2 weeks. Subsequently, patients with a complete recovery should be qualified for additional ivMP pulses in a 12-week protocol [6]. The main purpose of the additional therapy is to maintain the clinical improvement and minimize the inflammatory process. Nevertheless, its impact on QoL of the patients with DON has not been investigated until now.

Our study, performed on 6 patients with DON, showed no significant influence of the additional ivMP therapy on QoL. Simultaneously, an improvement in VA following the treatment was observed. A detailed analysis revealed that a decreased QoL after the additional treatment was associated with an exacerbation of diplopia and reduced motility of the eye muscles, which possibly deteriorated QoL, despite the improved VA. Furthermore, in some cases, the use of ivMP in high doses may lead to various side effects which can also interfere with QoL following the treatment [15], although generally it is considered to be highly efficient and mostly safe [16].

Results

The results of this study indicate that an additional 12-week ivMP treatment should constitute an integral part of the strategy in the management of DON. In terms of analysing a comprehensive therapy of DON, we should also consider involving rehabilitative surgery of the extraocular muscles. As demonstrated in our study, some patients consider the presence of diplopia to be a more relevant factor when assessing QoL, rather than improved VA. Therefore, strabismus surgery may have a positive impact on QoL.

To our knowledge, it is the first report evaluating changes in QoL in DON patient following the treatment with 12 pulses of ivMP. The main limitations of our study are its retrospective character and the small sample size. Nevertheless, the following conclusions may be drawn.

Conclusions

Including the additional 12 pulses of ivMP into combined therapy of DON may impact QoL. The assessment of QoL is, therefore, particularly important and should constitute an integral part of routine clinical practice. For some patients, diplopia or decreased eye motility become the main factor deteriorating QoL following the therapy, despite increased VA. The final evaluation of QoL should be performed after completing the entire therapeutic process, which involves surgical treatment to correct diplopia.

Following the first and second-line treatments of DON, a multidisciplinary and individual approach is necessary to maximize the potential improvement of signs and symptoms of the disease.

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

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ORIGINAL PAPER

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Efficacy of finisher files in the removal of calcium hydroxide paste from the root canal system – preliminary results

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ABSTRACT

Introduction. Successful endodontic treatment is affected by a number of factors associated with the disinfection and filling of the root canal. The chemical-mechanical root canal preparation consists in a thorough removal of any content from the pulp space, including inflamed pulp, bacteria, as well as canal filling materials.
Aim. The aim of the study was to analyse the efficacy of the XP-endo Finisher and the Brush-Finisher on the removal of a calcium hydroxide dressing.
Material and Methods. The study was conducted using extracted single-rooted human teeth prepared according to sample standardization. Calcium hydroxide with iodoform was inserted into the canals. After two weeks, canal cleaning was performed with the use of 2% sodium hypochlorite solution and both finisher files. A conventional endodontic needle and syringe (SNI) were used in the control group. Following rinsing activation, two projection radiographs were performed and uploaded to software developed specifically for the study. The graphic files were evaluated in terms of the remaining amount of dressing. In order to analyse whether the percentage of the canal area that remained untreated was statistically significant, the Kruskal-Wallis ANOVA test with Dunn's post-hoc test were employed.
Results. The intracanal dressing was most effectively removed in the XP-endo Finisher group (in both projections 96.32% and 91.35%), and its removal was considerably better than that in the control group ($p<0.0001$), although not significantly different from the Brush-Finisher group (89.68% and 81.85%).
Conclusions. Supplementary irrigant activation with either the XP-endo Finisher or the Brush-Finisher improved the removal of calcium hydroxide from the root canal walls.

Introduction

Prior to a root canal obturation, the intracanal dressing has to be completely removed from the canals in order to minimize its negative impact on the treatment prognosis [1]. A variety of different techniques have been used for the removal of material including stainless steel hand files, sonic

instruments and ultrasounds, rotary instruments, as well as lasers. Sonic and ultrasonic devices are reported to be more effective; however, until now there has been no universal consensus regarding the most effective method of removing intracanal calcium hydroxide ($Ca(OH)_2$) [2]. In endodontics, $Ca(OH)_2$ paste is the most commonly used intracanal medication, due to its antibacterial efficacy against the majority of endodontic pathogens and to its biocompatibility [3,4]. Nevertheless, residual parts the dressing which remain on canal walls, interact with some sealers, thus changing their physical properties, as well as reducing flow and setting time. Furthermore, the aforementioned residues also prevent the penetration of a sealer into the dentinal tubules, and may increase apical leakage of gutta-percha root fillings [1,5]. In fact, some clinicians consider abandoning the use of calcium hydroxide, since it cannot be completely removed from the entire canal system. However, the treatment of the infected canals, e.g. cases with the open apex and perforation or apexification procedures, still may require the use of this material. Therefore, the objective of the present study was to compare the effectiveness XP-endo Finisher and the Brush-Finisher files in removing calcium hydroxide paste from wide root canals.

Material and Methods

The Bioethics Committee of the Medical University of Lodz approved this experimental study (approval no. RNN/3988/19/KE of 12th December 2019). One hundred and fifty extracted human single-rooted teeth obtained from the Department of Dental Surgery at the Medical University of Lodz (Poland) and a private orthodontic dental clinic were radiographed (in two dimensions) in order to select those with single canals, curved up to 10 degrees (according to the Schneider' methods), without any canal fillings, calcifications or resorption. Thirty teeth (samples) were selected for the preliminary examinations. They were subsequently stored in 10% buffered formalin solution until use, and randomly divided into three groups: Group I (XP-endo Finisher, FKG Dentaire; La Chaux-de- Fonds, Switzerland), Group II (Brush-Finisher, MedicNRG, MedicNRG, Kibbutz Afikim, Israel) and Group III (conventional syringe and endodontic needle irrigation, SNI) consisting of 10 samples each as a preliminary study. The crowns were removed using a diamond disk to provide roots measuring 15 mm ± 1 mm in length. On the basis of the radiographs taken in two projections and the measurement of the apical foramen performed by means of hand files, wide root canals groups were established. If it was possible to wedge a hand initial file, larger than number 30 (≥0.3 mm), at the apical foramen, the canal was classified into a large root canal group (LC group, provided the middle and coronal part of the were loose (Figure 1). The ProTaper Next file number X3 (size 0.30 and taper: 7.5%; Dentsply Maillefer, Ballaigues, Switzerland), was introduced loosely (without a shaping procedure) up to the apical foramen to remove residual parts of the pulp and confirm the right classification of the canal width.

Following a thorough irrigation, using 2% NaOCl (5 ml) and 17% EDTA (5 ml), all canals were dried with paper points and filled with calcium hydroxide paste with iodoform (Calcipast

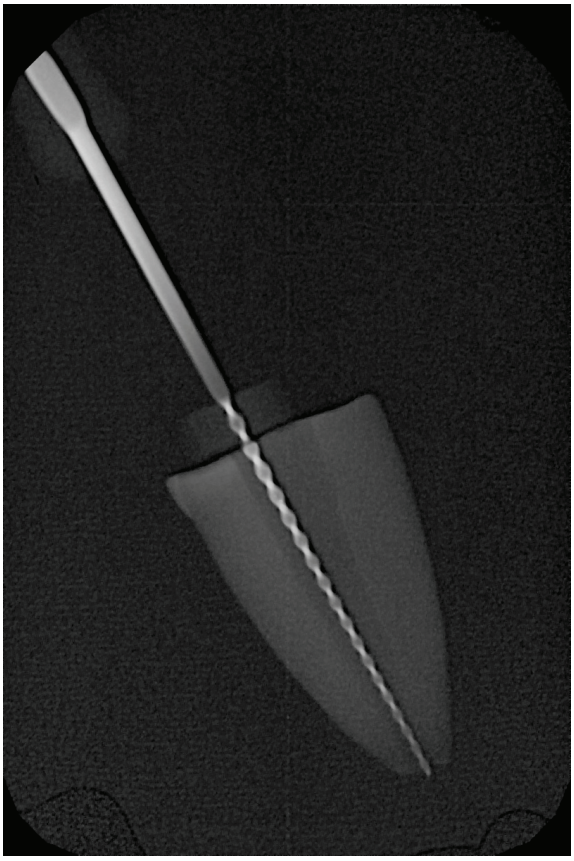

Figure 1. Classification of the canal as a wide root canal. If hand initial file was used first, larger than number 30 (≥0.3 mm), wedged at the apical foramen, the canal was classified as belonging to the large root canal group (LC group).

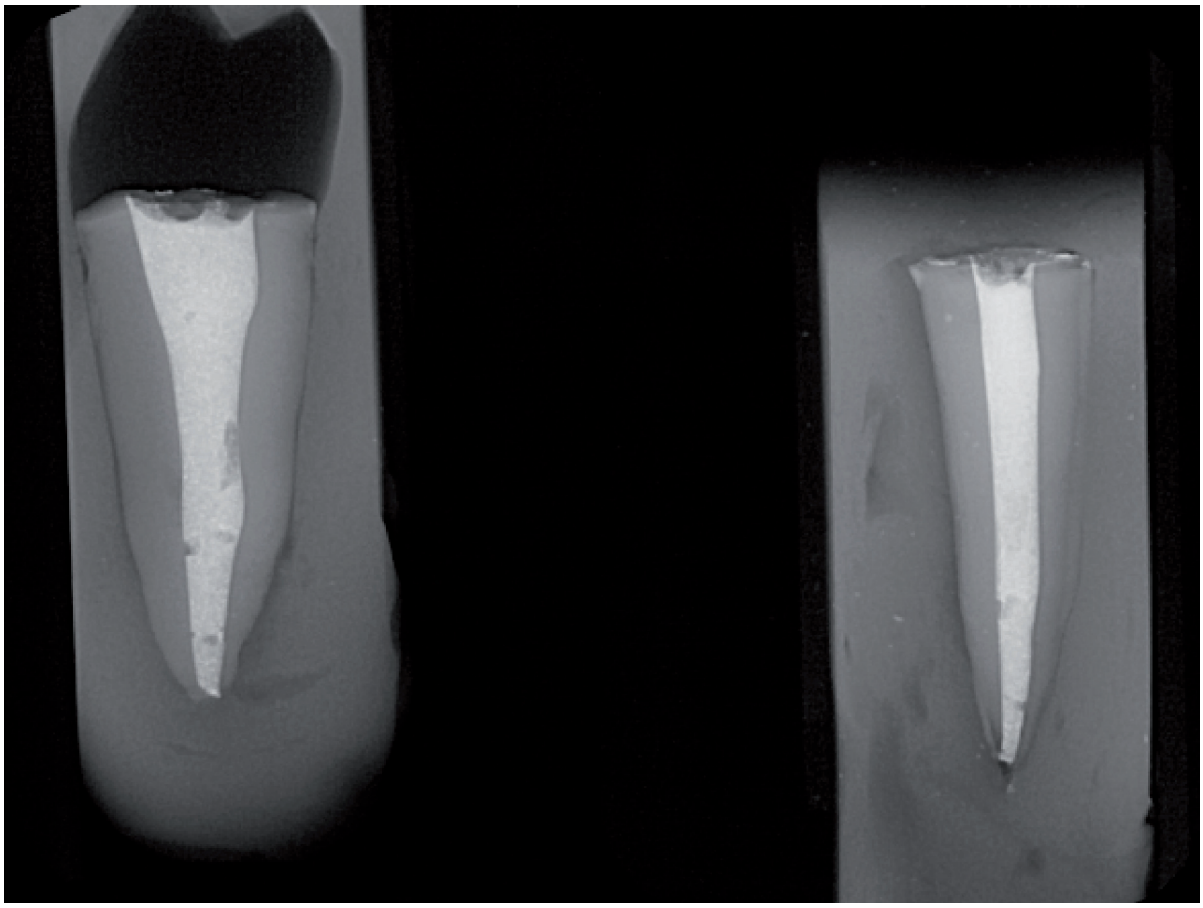

Figure 2. A buccolingual and mesiodistal radiograph of the canal filled with calcium hydroxide with iodoform paste

J, Cerkamed, Poland) (placed at 1 mm short of the apical foramen and withdrawn during dressing application) using an applicator. Additionally, the cavities were temporarily closed with materials based on zinc oxide and zinc sulphate. Next, each sample was placed into a silicone model in order to take two radiographs in the buccolingual and mesiodistal projection (GENDEX expertDC, 65kV, 7mA, 0.250 s, KaVo, Brea, USA) (Figure 2). If there was any void observed in the canal filling, the procedure was repeated until the sealing was acceptable. Subsequently, all samples were stored in an incubator at 37°C and 100% humidity for 14 days. Finally, the intracanal dressing was removed using three methods described below.

Group I

In the first group, the XP-endo Finisher files were used. The task was performed by one operator according to the manufacturer's instructions. The speed was 1000 rpm and the torque amounted to 1 Ncm. The working length was set using a plastic tube (a manufacturer's ruler). During the rotations, the file was worked in vertical movements in order to reach the full working length, and new portions of the solution were delivered continuously. The irrigant was heated up to 40°C and subsequently activated for approximately 15 seconds inside the canal. The procedure was repeated three times using 5 ml of the solution (working time: 1 minute ± 5 seconds). Decrease in the temperature of the solution (below 36°C) was minimized by a cyclic procedure of 15-second agitations and proper environmental conditions. The ambient temperature was approximately 20-26°C. The XP-endo Finisher files were cleaned from the residual parts of the dressing between individual work stages using abundant water irrigation and gauze, and their status was inspected. Finally, the "last drop" of the solution was aspirated - the canals were not dried using paper points, but allowed to dry for 3 minutes - and subsequently radiographed.

Group II

In the second group, the Brush-Finisher was applied, which is an additional instrument to the Gentlefile system. The task, as previously, was conducted by one operator according to the manufacturer's recommendations. The needle was inserted into the canal orifice, and the rinsing solution (5 ml) was introduced continuously (as in the previous group). Then, it was agitated by pecking motions up to the working length for approximately 1 minute ± 5 seconds. A short break provided every 15 seconds after activation in order to clean the Brush-Finisher and check the condition of the instrument. The remaining solution was aspirated and after a few minutes two radiographs of the canal were taken.

Group III

In the control group (SNI), endodontic syringes (with Luer-Lock, 5 ml) and needles (with a lateral opening, measuring 0.3 mm x 25 mm, EndoTop, Cerkamed, Poland) were used in a longitudinal and swivel movements. The rinsing was performed for approximately 1 minute by one operator. Finally, the remaining solution was aspirated and the radiographs were taken after 3 minutes.

In all the groups, one irrigating solution of the same volume, i.e. 5 ml of 2% NaOCl was applied. Each tooth specimen was coated entirely with silicone material and placed in a special holder, so as to protect it from the apical extrusion of the dressing, as well as from the operator's impact on the irrigation procedure. In group I, a special model bath was created to ensure a required body temperature. Following the dressing removal, two projection radiographs were repeated using a designed platform which ensured the same radiological position. Some samples with wide canals remained in excellent condition, and were further distributed to both groups where

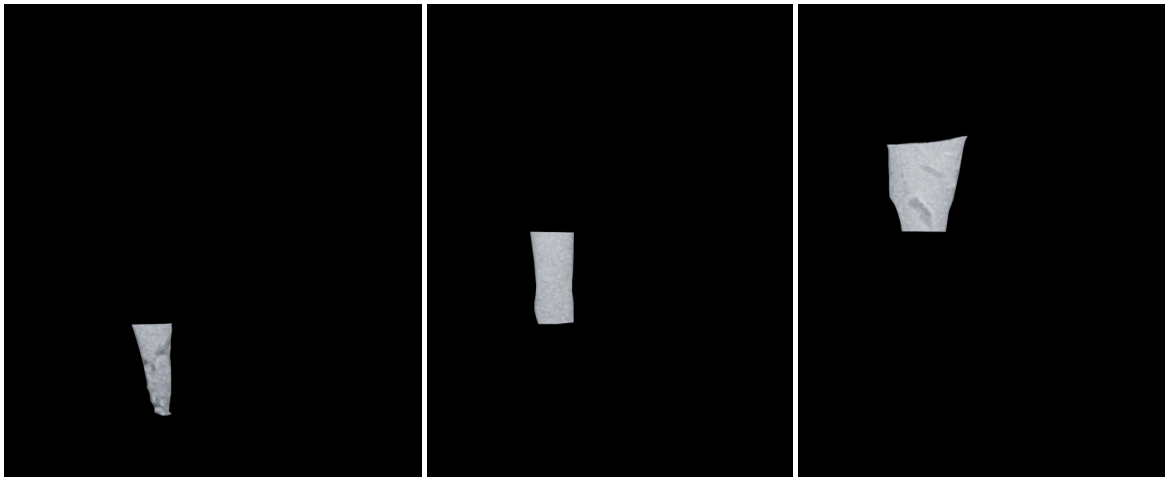

Figure 3. From left: a – The outlined and excited apical part of the canal space; b – The outlined and excited middle part of the canal space; c – The outlined and excited coronal part of the canal space

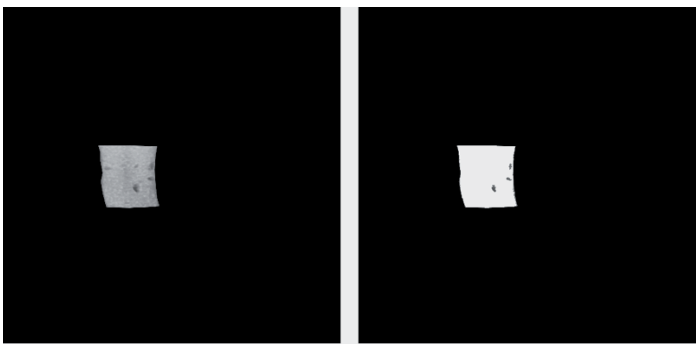

Figure 4. Analysis using custom-made software showing 95.5% of $Ca(OH)_2$ filling in the middle part of the canal. The first image (on the left) shows the middle part of the canal in the radiograph, the second image (on the right) presents pixels (PX) corresponding to the dressing residues

two different instruments were applied. All those canals were cleaned with an air abrasive technology, controlled radiographically and re-filled with a dressing. Subsequently, the images were uploaded to the graphic software (GIMP 2.8.22) and the root canals were divided into three parts: apical, middle and coronal. In order to make the image more legible for the analysis, graphic tools were applied to mark the canal space (Figure 3a, 3b, 3c). Next, new, custom-made software was applied to calculate pixel quantity (PX), corresponding to the dressing residues, and to convert the obtained values to a percentage of the canal surface area which remained untreated (Figures 4, 5, Table 1). Evaluations were performed twice, by one operator who was blinded to the study group.

Statistical analysis:

All the results were presented as a mean with a standard deviation and medians with interquartile range (IQR). The Kruskal-Wallis ANOVA test was used with the Dunn's post-hoc test in order to compare differences between >2 independent variables. A *p* value below 0.05 was considered statistically significant. All calculations were performed using Statistica 13 software.

Results

In terms of the entire canal length, the Kruskal-Wallis ANOVA test indicated that the SNI group presented poorer scores than the other groups

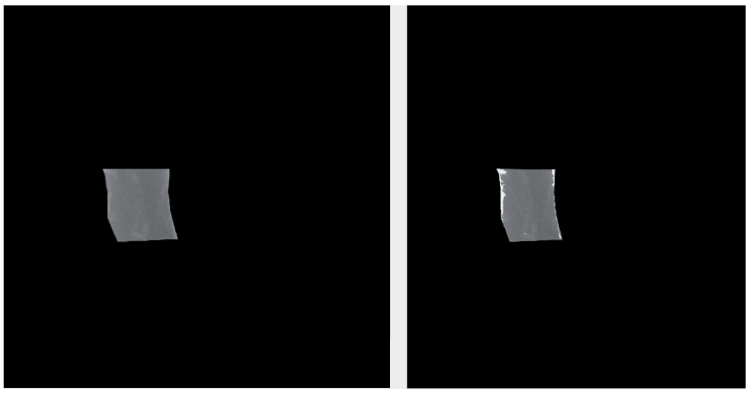

Figure 5. Analysis using custom-made software showing 3.54% of the remaining material in the middle part of the canal following the dressing removal. The first image (on the left) demonstrates the middle part of the canal in the radiograph, the second image (on the right) presents pixels (PX) corresponding to the dressing residues

Table 1. Table presents an excel sheet clipping of representative scores showing the process of calculation the percentage (2.22%) of unremoved calcium hydroxide dressing from the medial part of the canal after calibration procedure

Tooth code	Void	PX without voids	PX with voids	[%] canal	[%] apical	[%] middle	[%] coronal	[%] canal†	[%] apical†	PX middle*	PX middle after calibration**	PX middle remained***	[%] medial†	[%] coronal†
		Before $Ca(OH)_2$ removal						After $Ca(OH)_2$ removal						
4.3C	Yes	56609	51621	91.18	78.22	95.33	92.68	7.27	4.97	17939	17101.24	380	2.22	8.08

[%] canal/apical/medial/coronal, actual amount of $Ca(OH)_2$ filling expressed as a percentage; PX medial*, pixels of the image when the medial part was filled completely; PX medial after calibration**, pixels of the image taking into account that the medial part of the canal was filled incompletely (with voids); PX medial remained***, pixels of the image of the remaining $Ca(OH)_2$ in the medial part of the canal; [%] medial†, actual amount of the remaining $Ca(OH)_2$ in the medial part of the canal expressed as a percentage.

(*P*< 0.0001) (Table 2), both with regard to the buccolingual, as well as the mesiodistal projection. In the mesiodistal projection, the median residue dressing amounted to 77.29% in the SNI group, 22.68% in the Finisher-brush group and 11.75% in the XP-endo Finisher group (*P*=0.0001). None of the investigated groups achieved 100% cleaning efficacy in removing calcium hydroxide from the root canal walls. The XP-endo Finisher removed the largest amount of the canal dressing and its results were superior in the apical and middle portions of the canal (in the BL=buccolingual projection 96.32% and 96.92%; and in the MD=mesiodistal projection 91.35% and 92.79%), as compared to the coronal portion (in the BL=buccolingual dimension 91.3%, and in the MD=mesiodistal projection 87.63%) (Table 3). In the Finisher-brush group, the observed scores were close to those of the XP-endo Finisher files, without a significant difference (in the BL projection 89.68% and 90.01%; and in MD projection 81.85% and 79.46%) (Table 3). In all the groups, the largest amount of the remaining intracanal dressing was found in the coronal thirds (Fig. 6a, Fig. 6b). In both Finisher groups, the difference was statistically insignificant (Table 4).

Table 2. Remaining $Ca(OH)_2$ [%] in the whole canal length concerning the control group, the Brush-Finisher and XP-endo Finisher group in buccolingual and mesiodistal projection

Canal %	SNI		Brush-Finisher		XP-endo Finisher		*P*-value
	B-L	M-D	B-L	M-D	B-L	M-D	
Median	63.81	77.29	12.46	22.68	5.05	11.75	<0.0001
1st quartile	55.36	64.45	9.44	14.00	1.83	6.00	
3rd quartile	66.05	81.68	19.01	32.69	15.83	26.02	
Min.	32.89	37.78	5.13	12.30	0.30	0.40	
Max.	76.90	91.10	36.34	61.87	22.66	39.75	

Data was presented as medians (squares) with interquartile range (boxes) and min-max values (whiskers). The Kruskal-Wallis ANOVA test was used.

Table 3. Residual $Ca(OH)_2$ [%] in the SNI, XP-endo Finisher and the Brush-Finisher group in the buccolingual and mesiodistal projection in the apical, middle and coronal portion of the canal

Dimension	Canal portion	SNI	X-Pendo Finisher	Brush-Finisher	p-value
Buccolingual	apical %	32.99	3.68	10.32	0.0061
	middle %	56.39	3.08	9.99	0.0001
	coronal %	78.22	8.70	16.15	<0.0001
Mesiodistal	apical %	54.26	8.65	18.15	0.0058
	middle %	74.57	7.21	20.54	0.0001
	coronal %	71.56	12.37	23.18	0.0001

Data were presented as medians. The Kruskal-Wallis ANOVA test was used.

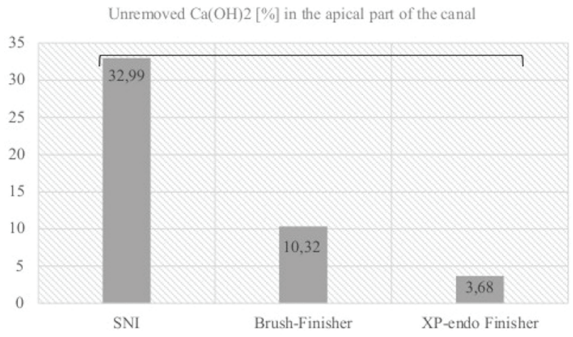

Figure 6a. The chart presents results in the apical third of the canal in all groups (buccolingual projection). The significant difference is marked between the tested groups. The Kruskal-Wallis ANOVA test was applied with the Dunn's post-hoc test

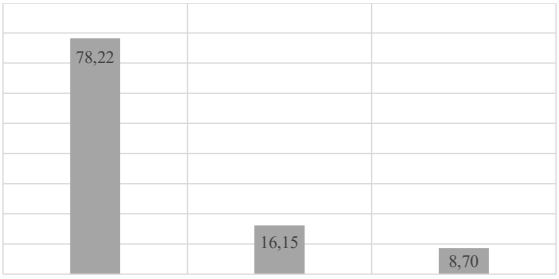

Figure 6b. The chart presents the results in the coronal third of the canal in all groups (buccolingual projection). The significant difference is marked between the tested groups. The Kruskal-Wallis ANOVA test was applied with the Dunn's post-hoc test

Table 4. A comparison of the significant differences between the SNI, XP-endo Finisher and the Brush-Finisher group in the buccolingual and mesiodistal projection in the apical, middle and coronal portion of the canal

Buccolingual dimension		X-Pendo Finisher			Brush Finisher		
		apical %	middle %	coronal %	apical %	middle %	coronal %
SNI	apical %	0.0049	-	-	ns	-	-
	middle %	-	0.0001	-	-	0.0144	-
	coronal %	-	-	0.0000	-	-	0.0029
X-Pendo Finisher	apical %				ns	-	-
	middle %				-	ns	-
	coronal %				-	-	ns

Mesiodistal dimension		X-Pendo Finisher			Brush Finisher		
		apical %	middle %	coronal %	apical %	middle %	coronal %
SNI	apical %	0.0041	-	-	ns	-	-
	middle %	-	0.0001		-	0.0123	-
	coronal %	-	-	0.0001	-	-	0.0029
X-Pendo Finisher	apical %				ns	-	-
	middle %				-	ns	-
	coronal %				-	-	ns

The Dunn's post hoc test was used.

Discussion

The aim of this study was to compare the impact of the XP-endo Finisher, the Brush-Finisher, and the use of a syringe and an endodontic needle on the removal of an intracanal dressing. The null hypothesis that the irrigation groups do not differ from each other in removing calcium hydroxide from wide canals was rejected. The use of XP-endo Finisher and the Brush-Finisher demonstrated a better efficacy in removing the dressing, with 94.95% and 87.54% of the calcium hydroxide removed, respectively. These results were applicable to the buccolingual dimension and did not differ significantly from each other. In contrast, they were considerably better than the results achieved in the control group (36.19%, $P<0.0001$). Furthermore, in the mesiodistal projection, the XP-endo Finisher and the Brush-Finisher also removed a large portion of the canal dressing (88.25% and 77.32%). Additionally, their results were significantly better than in the control group (22.71%, $P=0.0001$). These findings are in accordance with the observations made by Uygun et al. [6], who claim that the TRUShape 3D Conforming File and the XP-endo Finisher can be beneficial in removing calcium hydroxide from root-canal walls via continuous irrigation. In the present study, the XP-endo Finisher was compared to the Brush-Finisher.

In the literature, we found only two studies assessing Brush-Finisher's efficacy. Firstly, Neelakantan et al. [7] conclude that the use of the Finisher GF Brush (i.e. Brush-Finisher) improved the debridement of canals. Therefore, in our research, we confront files which both work at high speed (XP-endo Finisher: 800-1000 rpm and Brush-Finisher: 6500 rpm) and rotate in one direction. Moreover, their application in the course of the cleaning procedure is similar. According to the manufacturer, the files go in up-and-down movements with an amplitude of 7-8 mm for approximately 1 minute. Both instruments are made of different material (the XP-endo Finisher is made of Ni-Ti alloy, whereas the Brush-Finisher is made of stainless steel) and have a different design, thus, we may compare their impact on the effectiveness of $Ca(OH)_2$ removal. Irrigation protocols were highly standardized, performed with an exact positioning of tips, identical flow rates and activation times, executed by one person (an endodontist) and the analysis was performed twice with a double blinded test at a three-week interval. Interestingly, as shown in Table 3, the activation with the XP-endo Finisher and the Brush-Finisher resulted in a similar pattern of the intracanal dressing removal, as described by De Deus et al. [8]. The coronal portion of canals was cleaned less effectively than the other areas in both groups, but the difference was not statistically significant ($P>0.01$).

The material used in the study included extracted, single-rooted (with a single canal) human teeth, verified with radiographs and measurements of the apical foramen. Cases where a hand file larger than 30 (≥0.3 mm) was possible to be wedged at the apical foramen, were included into the wide root canal group, if both the middle and coronal portion of the file were loose. Apart from the advantages of a biological material and a great emphasis on the standardization of samples, this material possibly presents certain drawbacks, e.g. differences in the extent of the canals (e.g. taper, 3D dimensions). This is a common disadvantage of using extracted teeth; however, it does not exclude the suitability of the material. To address this, we created several groups (e.g. the XP-endo Finisher, the Brush-Finisher, and the control group), each of them consisting of a representative number of samples resulting from the preliminary research (thus, forming the separate wide and narrow canal groups). In the literature, there are numerous studies which involve the artificial groove model [9], whereby such a pre-drilled groove is assumed to imitate the canal isthmus and irregularities. According to the model described by Lee et al. [10], it is 4 mm long, 0.5 mm deep and 0.2 mm wide. Nevertheless, this model has not been chosen for our study, since it does not represent the complexity of the physiological root canal anatomy [9,11] and does not correspond to the anatomical conditions present in wide canals.

In addition, apart from the high magnification image analysis, the artificial groove model allows to evaluate the presence of a dressing still in two dimensions (length and width). In other studies, cone-beam computed tomography is used for the calculation of remaining $Ca(OH)_2$. This technique enables volumetric analysis, however, it may also be based on particular scans of the root canal sections and uniform grayscale threshold to visualize and quantify the volume of the residual $Ca(OH)_2$ material [12]. Nowadays, MicroCT is one of the most effective methods, although remains still quite unavailable [13]. In this study, we provided the same protocol conditions taking the canal radiographs in two projections following the dressing insertion and after its removal. Image acquisition was standardized by means of repetitive sample positioning (with the use of a custom-made platform) and a uniform radiological setting. Moreover, new, custom-made software was used to calculate pixels quantity (PX), corresponding to the dressing residues, and finally to convert them to the percentage of the canal surface area which had not been cleaned. In fact, similar software was used in another study assessing biofilm-mimicking hydrogel removal [14].

Since numerous articles indicate serious difficulties in removing intracanal dressing from the root canal system, in our study we decided to use calcium hydroxide with iodoform as endodontic medication [9,15]. Firstly, it is vital to bear in mind that a thorough debridement of the apical part of the canal constitutes an exceptional impediment [16]. Another issue is obtaining optimal cleanliness of oval or very wide canals, as the use of mechanical instrumentation might be limited due to the necessity of preserving root tissue [17]. Nevertheless, it is crucial that the canal system be hermetically filled with an intracanal dressing. Thus, we inserted calcium hydroxide paste with an applicator, beginning the insertion from the apical to the coronal part of the canal, and its homogeneity was confirmed radiologically. Any smallest void visible in the canal path was accounted for before and after pixel calculation. This resulted in the objective outcome variable, which constitutes the percentage of the remaining calcium hydroxide paste. A similar technique was employed in other studies, e.g. assessing cleaning efficacy during root canal retreatment [18,19].

The most commonly used method of removing $Ca(OH)_2$ is a combination of abundant rinsing and the preparation with a master apical file (MAF) of the entire working length [20]. According to the literature, one of the most effective methods of removing dressing from the apical third of the canal is ultrasonic rinsing activation [21]. On the other hand, Wigler et al. [22] compared the ultrasonic technique with the XP-endo Finisher activation lasting 1 minute and achieved comparable results. Moreover, there was also no statistically significant difference between the XP-endo Finisher and ultrasonic activation in the study by Leoni[23]. In our study, the best efficacy of removing the dressing from the apical part of the canal was obtained in the XP-endo Finisher group in both dimensions (96.32% and 91.35%). Additionally, similar results were achieved in the Brush-Finisher group (89.68% and 81.85%) without any significant difference. In fact, the XP-en-

do Finisher is very flexible and it can expand during rotation. Additionaly, the design of the Brush-Finisher's may affect the cleaning efficacy due to splitting its filaments in the course of application and reaching the canal surface in the apical portions. This, in turn, is in accordance with the results of Hristov and Gateva [24], who compared six irrigation protocols eliminating bacteria in root canals of immature permanent teeth. They reported that the reduction in the number of colony-forming units was significantly superior in the XP-endo Finisher (and EDTA) group and the Gentlefile brush (and citric acid, i.e. Brush-Finisher) group, which may support the findings in the present study.

Since the 1-min operation time was not sufficient for the effective removal of $Ca(OH)_2$ from the canals in this study, longer activation periods should be verified before formulating final, probably more comprehensive, conclusions. We present preliminary results with regard to the cleaning efficacy of large canals, thus, it is necessary to perform comparable research concerning narrow canals in the immediate future.

Perspectives

Concluding, none of the methods used in the study was able to entirely remove $Ca(OH)_2$ from wide root canals. However, considering the limitations of this study, it can be concluded that the XP-endo Finisher and the Finisher-brush are beneficial in removing calcium hydroxide from the root canal walls.

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Contribution
AK: concept design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and manuscript review; PK: Contributed to: data acquisition, data analysis, statistical analysis, manuscript editing

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

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ORIGINAL PAPER

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Worry and depression levels among patients with type 1 diabetes mellitus. The mediating role of illness acceptance

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ABSTRACT

Aim. The purpose of the conducted study was to explore the role of cognitive processes, such as habitual worry, with regard to depressive mood in patients with type 1 diabetes mellitus (T1DM), as well as the significance of illness acceptance in the form of personal asset and mediator in relation between worry and depression.

Material and Methods. The study involved 229 participants diagnosed with T1DM, who completed a set of self-description questionnaires. Assessment methods included The Anxious Thoughts Inventory (AnTI), The Acceptance of Illness Scale (AIS) and The Center for Epidemiologic Studies Depression Scale (CES-D).

Results. The results demonstrate that worry is positively correlated with depression. What is more, the relationship between habitual worry and depression was mediated through illness acceptance.

Conclusions. The impact of habitual worry on mental health in T1DM cannot be ignored. Moreover, the observed dependence suggests that depressive mood present in individuals with T1DM may persist, since overly worried patients do not accept their own illness. Strengthening patients' acceptance of their condition and bringing up the topic of worry in the course of diabetes education, can be a powerful tool in depression prevention. Nevertheless, further research is necessary.

Introduction

Type 1 diabetes mellitus (T1DM) is an extremely complex chronic condition, which requires patients to adjust to the specific treatment regime and numerous restrictions. Daily tasks include proper medication intake, regular glycaemic control, a balanced diet, and physical activity performed during basic everyday activities [1]. Individual approaches to self-management can

significantly affect patient s' current and future health status [2]. Poor self-care increases the risk of developing specific diabetic complications [3] and entails a higher risk of premature death [4], whereas good adherence results in a decreased risk of hospitalization [5] and in better general health outcomes [6]. Among many factors affecting the quality of diabetes treatment, a patient's decisions and motivations form solid foundations of the entire treatment process. As a result, patients not only experience constant fear for their lives, but also bear a great responsibility for each decision they make in the face of the chronic condition which may lead to overwhelming emotional distress, experienced on a daily basi s [7–9].

The c ross-national DAWN2 study [10], involving over 8 thousand adults diagnosed with T1DM, described diabetes as a 'significant physical and psychological burden for many individuals'. In fact, d iabetes is strongly associated with comorbid psychological and psychiatric issues [9], and the data indicate that the onset of the illness increases the possibility of depressive symptoms [11,12]. Severe consequences of comorbidity of depression in T1DM have become a crucial aspect and have been the focus in numerous scientific debates, due to their impact related to the deterioration of patient self-care and diabetes management [13,14]. Hence, the American Diabetes Association recommendations [15] clearly indicate the continuous need for depression to be a valid factor, present in clinical practice, as well as in future diabetology-related research.

The mainstream theory, accounting for the mechanisms responsible for the co-occurrence of depression in the diabetes population, refers to the idea of 'chronic stress'. In fact, prolonged stress associated with the disease-related restrictions may result in an extensive activation of the hypothalamic-pituitary-adrenal axis, and hence lead to an increase in cortical growth. This, in turn, is described as a pathway that helps to interpret the clinical relationship between T1DM and depression [16]. However, in order to better understand the impact of chronic disease on mental health, it is vital to address the following questions: why does this problem only affect some patients, and what makes the remaining group manage well with chronic stress caused by the disease?

Adaptation to a chronic disease constitutes a continuous and extremely complex process [17–19]. There are numerous theories explaining the course and components of the adaptation pathway. Nevertheless, the common denominator of these theories is the role of metacognitive processes which constitute the most crucial factors involved in the adaptation process. The most important theoretical approaches to date refer to the cognitive functions involved in adaptation to the disease as cognitive effort [20], coming to terms with the illness [21], finding benefits [22], illness perception [23] evaluation of the stress transaction [24], etc. Thus, the onset of depression can be perceived as a consequence of choosing a wrong metacognitive strategy as a form of coping with the disease-associated stress.

In the present paper, habitual worry is interpreted as a metacognitive mechanism activated due to the stress associated with everyday challenges in T1DM. Moreover, in its pathological form, it is related to the perseverative negative thinking loop, which constitutes an inadequate coping strategy leading to an impaired emotional self-regulation [25]. The adaptation of the metacognitive model of pathological worry to account for the severity of depression in T1DM patients seems theoretically promising for two reasons. Firstly, similar research models obtained statistically significant results [26–28], and s econdly, chronic disease, such as diabetes, is extremely dynamic in its course and affects many levels of patients' daily life functioning. Nevertheless, successful treatment is 95% dependent on patients' self-care behaviours [29]. Therefore, it appears that worry could be an integral part of chronic illness treatment in the form of cognitive strategy, which helps dealing with uncertainty, volatility and unpredictability, as well as with planning and self- care in diabetes treatment.

The metacognitive model differentiates two kinds of worry: (1) the first type involves coping with daily life challenges, whereas (2) the second one, also referred to as 'meta-worry', includes negative evaluation of worry [30]. Although positive observations regarding worry are considered a common and a non-pathological strategy of dealing with problems, e.g. 'If I worry about my blood glucose level, I will always be adequately prepared', negative ones seem to increase the

sensitivity to threatening stimuli, thus, hindering individual adaptive coping strategies due to deep, looped, and pathological worry, e.g., 'My worry is uncontrollable, I am going to lose my mind' [31].

According to the literature, in contrast to the phenomenon of worry, acceptance is often referred to as a special agreement between patient and the disease [32], a change of orientation towards positive aspects of everyday life [33], and a positive self-perception [34]. By definition, illness acceptance is a personal resource reflect ing the attitude of full understanding and a sense of self-worth, despite the current state of health, disability or dependence [35]. As a personal resource, acceptance constitutes a stable feature, a kind of prism that can set the course for dealing with the chronic disease-specific stress transactions [36], for instance, as an examination and correction of the inner coping mechanisms and behaviour patterns [37]. Research concerning the issues of health psychology has indicated that acceptance of illness promotes better adaptation to the disease [35], affects patients' dispositional optimism [38] and quality of life assessment [39,40]. Similar observations were made in the studies involving groups of patients with type 1 or type 2 diabetes mellitus (T2DM) [41–43].

Aim

The first objective of this study was to test the hypothesis of the relationship between habitual worry and the level of depression among patients with T1DM. The second aim was to investigate the hypothesis regarding the mediating role of illness acceptance in view of habitual worry and the depressive mood in T1DM patients.

In this particular study, the following two hypotheses were formulated: (1) T1DM patients present a higher level of habitual worry and of depressive mood, (2) acceptance of illness, as a personal resource, mediates the relationship between worry and depressive mood.

Material and methods

The study was conducted in 2018 and 2019, in the pre-COVID-19-pandemic period, at the Department of Internal Medicine and Diabetology, Poznan University of Medical Sciences (PUMS), among in-patients suffering from T1DM. The study was questionnaire-based (see description below). Patients who agreed to participate in the study received a set of 3 questionnaires as well as a demographic survey collecting data, such as: gender, disease duration, marital status, education, residence and employment, all in an envelope. Completed questionnaires were collected in a secure box at the PUMS Department of Diabetology, which ensured complete anonymity of the participants. The project was approved in November 2018 by the PUMS Bioethics Committee (Resolution number 1123/18).

The inclusion criteria for the study were as follows: age over 18, disease duration over 3 months, ongoing insulin therapy and a written informed consent to participate in the study. The criteria for exclusion from the study were: previous acute infections, surgery or other severe complications within the last 3 months, pregnancy, coexisting diseases, such as heart, lung, kidney or liver failure, cancer, confirmed mental illness or mental disorders preventing the completion of the questionnaire.

Assessment methods included The Anxious Thoughts Inventory (AnTI), The Acceptance of Illness Scale (AIS), as well as The Center for Epidemiologic Studies Depression Scale (CES-D).

The Anxious Thoughts Inventory (AnTI) developed by Wells [44], was used to assess generalized worry. AnTI is a 22-item self-report scale, assessing three dimensions of worry: social worry, health worry, and meta-worry. The scale was created to emphasize the differences between worry (concerns about daily life) and metacognitions about worry (concerns about worry and cognitive functioning) [31]. Participants are asked to use a four-point Likert scale to respond to the test items. The total score represents the sum of all the provided responses [44]. Psychometric properties of AnTI were reported to be satisfying, both in the general population, as well as in clinical trials. The reliability of AnTI was sufficient for the purpose of the study - *alfa* Cronbach's=0. 96 (see Table 3).

The Acceptance of Illness Scale (AIS), developed by Felton, Raveson, and Hinrichsen [35], was used to evaluate the acceptance of illness. The scale is presented according to the Polish adaptation by Juczyński [45], and measures the inten-

sity of successful disease acceptance, despite its association with disability, dependency or sense of worthlessness. Originally, AIS was applied by the authors as part of psychological interviews assessing the degree of psychological adjustment in adult patients with chronic conditions. The scale consists of 8 statements, describing the consequences and limitations resulting from a disease. The participants use a five-point scale to respond to the test items: starting from 1 – I strongly agree, to 5 - I strongly disagree. The total score represents the sum of all the provided responses. A high score indicates acceptance of one's own medical condition, whereas a low score reflects a lack of acceptance and adaptation to the disease [35]. The reliability of AIS was sufficient for the purpose of the study - *alfa* Cronbach's=0. 85 (see Table 3).

The Center for Epidemiologic Studies Depression Scale (CES-D) developed by Locke and Putnam [46], was used to determine depression levels. The Scale was provided according to the Polish adaptation created by Ziarko, Kaczmarek and Haładziński [47]. It is a brief self-report tool, designed to assess "the current level of depressive symptomatology, with emphasis on the affective component, depressed mood" in the general population (p.385) [46]. It consists of 20 statements, describing the frequency of affective, cognitive or somatic depression signs, experienced during the last week. Participants use a four-point scale to respond to the tool items: where 1 means "Rarely or not at all", and 4 - "Mostly or all the time". A high score reflects a greater frequency of depression symptoms occurrence. The reliability of CES-D was sufficient for the purpose of the study - *alfa* Cronbach's=0. 88 (see Table 3).

The collected questionnaires and surveys were verified for completeness and subsequently entered into the statistical package IBM® SPSS® (version: 25.0 license 5725-A54) in order to extract statistical information. The collected data were analysed in three steps. Firstly, simple Pearson's r correlation coefficients were calculated to assess the relationship between generalized worry, acceptance of illness and depression. Secondly, simple Pearson's r correlations coefficients were calculated to evaluate whether the age of patients affected the obtained results. Thirdly, a mediation analysis was performed according to the method suggested by Preacher and Hayes [48], i.e. to test the hypothesis regarding the mediating role of acceptance in the relationship between worry and the level of depression. In the analysis of mediation, a resampling procedure was conducted, with five thousand repetitions.

Results

The final study sample involved 229 volunteers diagnosed with T1DM. All respondents agreed to participate in the study and completed a set of self-description questionnaires. Women and men who participated in the study were of similar age and had been suffering from diabetes for a similar period of time (see Table 1).

Other demographic data have been presented in Table 2. The studied population was dominated by patients who were married 97 (42. 4%), with tertiary education 62 (27. 1%), who live in the countryside 69 (30. 1%) and who were professionally active 133 (58. 0%).

In the course of the analysis of the relationship between worry and depression, it was found that the level of generalized worry correlated positively with the level of depression. The obtained correlation coefficients ranged from r=-0.16** in terms of the relationship between worry and a sense of well-being, and up to r=0.73** regarding the relationship between worry and depressive mood. Generalized worry correlated negatively with illness acceptance (see Table 3).

It was also assessed whether the age and the duration of the disease were related to the vari-

Table 1. Characteristics of the study group

Variable	Participants (n=229)				Test t		
	Men (n=88)		Women (n=141)				
	Mean	±SD	Mean	±SD	t	df	p
Age	39.36	10.33	30.18	10.36	-0.127	185.213	0.889
Disease duration	10.42	7.75	11.57	7.61	1.089	183.030	0.274

Source: in house materials.

Table 2. Demographic characteristics of the study group

Variables		n	%
Marital status	Married	97	42.4%
	Engaged	25	10.9%
	Cohabitation	12	5.2%
	Relationship	24	10.5%
	Single	57	24.9%
	Other	14	6.1%
Education level	Primary	14	6.1%
	Basic vocational	30	13.1%
	Secondary vocational	45	19.7%
	Secondary	31	13.5%
	Post-secondary	23	10.0%
	University student	24	10.5%
	University Graduate	62	27.1%
Residence	Rural	69	30.1%
	Urban	157	68.6%
Occupation	Working	133	58.0%
	Not working	71	31.1%
	Retired	24	10.5%

Source: in house materials.

ables included in the study (see Table 4). There was a weak correlation between age and depression $r = 0.15*$ and one of its components - somatic symptoms $r = 0.14*$.

Figure 1 shows the graphic presentation of a simple mediation model investigated in this study. The presented material reveals how habitual worry and its components affects depressive mood through illness acceptance. Path *a* (left arrow) represents the effect of habitual worry on illness acceptance, whereas path *b* (right arrow) is the effect of illness acceptance on depression neglecting the effect of worry. Path *c* (above mid-

Table 4. Pearson's correlation coefficient between age, disease duration and the analysed variables

	Age	Diseaseduration
Habitual worry	0.04	-0.03
Social worry	-0.07	-0.06
Health worry	0.12	-0.02
Meta-worry	0.07	0.01
Depression	0.15*	0.07
Depressive mood	0.13	0.09
Well-being	0.03	0.03
Somatic symptoms	0.14*	0.01
Attitude towards people	0.10	0.07
Illness acceptance	-0.03	-0.08

* p < 0.05. ** p < 0.01

Table 3. Descriptive Statistics, Cronbach's Reliability Coefficients, and Correlations Between Variables

	Range	Mean	±SD		z	1	1a	1b	1c	2	2a	2b	2c	2d
Habitual worry	22.00-76.00	40.63	11.88	0.96	0.09**									
Social worry	9.00-35.00	16.87	5.21	0.88	0.09**	0.91**								
Health worry	6.00-22.00	10.94	3.69	0.84	0.13**	0.79**	0.55**							
Meta-worry	7.00-26.00	12.82	4.38	0.86	0.11**	0.95**	0.80**	0.67**						
Depression	0.00-49.00	17.48	10.42	0.88	0.14**	0.64**	0.55**	0.53**	0.63**					
Depressive mood	0.00-21.00	5.31	5.14	0.90	0.16**	0.73**	0.63**	0.58**	0.72**	0.89**				
Well-being	0.00-10.00	6.01	2.13	0.74	0.14**	-0.16*	-0.13*	-0.09	-0.16*	0.18**	-0.11			
Somatic symptoms	0.00-20.00	5.21	4.77	0.85	0.14**	0.59**	0.49**	0.53**	0.58**	0.90**	0.79**	-0.04		
Attitude towards people	0.00-6.00	0.94	1.39	0.77	0.37**	0.45**	0.45**	0.30**	0.41**	0.66**	0.58**	0.10	0.53**	
Illness acceptance	8.00-40.00	26.67	7.35	0.85	0.08**	-0.38**	-0.36**	-0.30**	-0.34**	-0.42**	-0.48**	0.05	-0.39**	-0.27**

* p < 0.05. ** p < 0.01

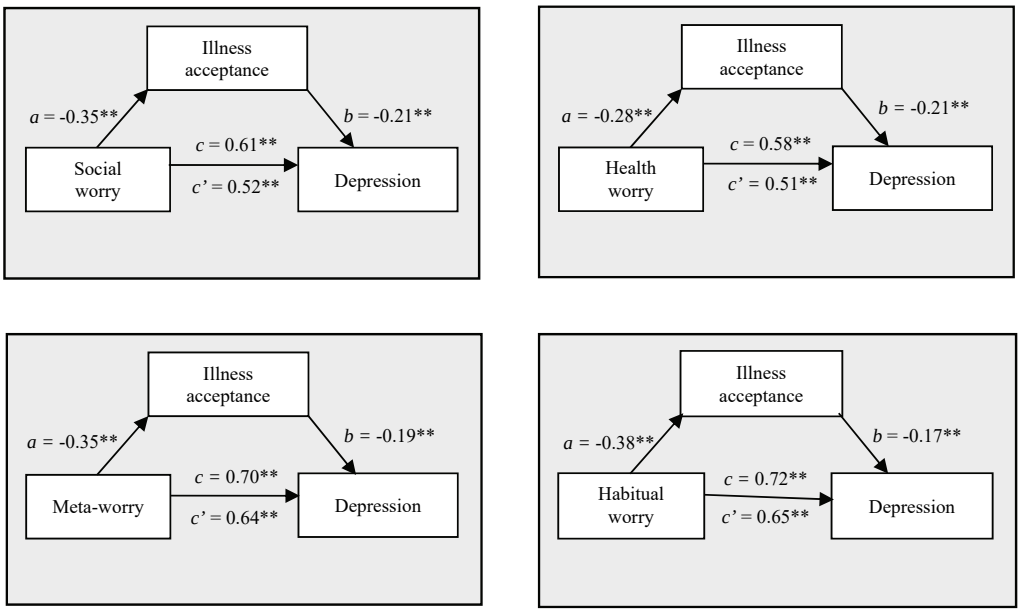

Figure 1. Mediating role of illness acceptance (M) to habitual worry (X) and depression (Y). * p <0.01, ** p <0.05

dle arrow) shows the indirect effect on depression through illness acceptance, whereas path *c'* (below middle arrow) demonstrates the direct effect on dependent variables. Paths *a, b, c* and *c'* present regression coefficients.

The relationship between the independent variable of habitual worry and the dependent variable of depression was mediated through the mediator, i.e. the acceptance of illness. Acceptance demonstrated to be a mediator for both the general level of worry and its single components (social worry, health worry, and meta-worry), as well as for the severity of depression. The observed dependence suggests that depressive mood in people with type I diabetes may persist, since excessively worried patients do not accept their own illness. The observed mediations are partial, which means that other factors also mediate the investigated relationship (see Table 5).

Discussion

The results obtained in the current study confirmed the hypothesis regarding the correlation between worry (and its individual components: social worry, health worry, meta-worry) and depression among patients with T1DM. First of all, this supports the data found in literature that worrying is a phenomenon commonly present not only in anxiety disorders, but also in depression [25]. Furthermore, the study found that the nature of worry seems to reflect rumination thinking style in severe depression in patients suffering from diabetes. In particular, following the metacognitive model of emotional disorders as a response to negative circumstances, the selected coping strategy can be negatively evaluated on the metacognitive level [31]. Despite the fact that the scientific data [27] suggest that

Table 5. Mediating role of illness acceptance (M) with regard to habitual worry (X) and the level of depression (Y)

	Paths				Model summary			Sobel test		95% CI	
	a	b	c	c'	R^2	F	p	z	p	Lower	Upper
Habitual worry	-0.38**	-0.17**	0.72**	0.65**	0.53	129.12	<0.001	3.14	0.017	0.02	0.13
Social worry	-0.35**	-0.21**	0.61**	0.52**	0.41	81.90	<0.001	3.36	<0.001	0.02	0.14
Health worry	-0.28**	-0.26**	0.58**	0.51**	0.41	79.97	<0.001	3.24	0.001	0.03	0.14
Meta-worry	-0.35**	-0.19**	0.70**	0.64**	0.53	129.54	<0.001	3.25	0.001	0.02	0.13

* p < 0.05. ** p < 0.01

a depressive thinking style is rather associated with ruminations (orientation to the past personal loss, accidents, failures, etc.) than with worry (future-problem orientation, in example: "What if…?"), the meaning of worry and its components in terms of chronic disease cannot be neglected. Particularly, since diabetes management is primarily based on planning and anticipating current issues and future challenges. In this sense, worry constructs a desirable, significant form of adaptive cognitive mechanism prepar ing the individual for possible danger, as well as takes part in the decision making process [49]. Unfortunately, constant, uncontrollable, or too frequent preparatory system activation is considered maladaptive due to the emotional and information - processing disruption, resulting in individual high emotional costs [50], and may reflect symptoms of mood disorders. According to Matthews and Funke [51], 'worry relates to general tendency towards various forms of negative self-referent thinking' (p.64), e.g. in the sense of self-incompetence, catastrophic thinking loop, and/or avoidance coping strategies of choice. In daily management of diabetes, maladaptive worry may explain withdrawal from active treatment and patients' non-adherence. Recent studies also emphasized the role of metacognitive processes, such as worry, which are involved in the onset of a patient's depression. Moreover, Ziarko, Jasielska, and Mielcarek [28] studied the group of 210 hospitalized patients with rheumatoid arthritis, and found that depressive mood was associated with the habitual meta-worry and worry.

The current study demonstrated that the age of T1DM patients affects depressive mood and somatic symptoms in a limited although statistically significant way. This result is similar to the previous data obtained in large-scale studies [10].

The obtained data confirmed the hypothesis with regard to the mediating role of acceptance of illness between worry and depressive mood, which is consistent with the literature review [25, 41-43]. The test results indicate that the lack of acceptance may constitute a kind of vulnerability, exacerbating the depression. Conversely, high acceptance can help patients adapt well to the challenges of diabetes and provide a protective barrier against the development of mood disorders.

The current recommendations of the American Diabetes Association [15] promote the involvement of people qualified to provide psychological assistance in the course of diabetes management. The results obtained in the present paper indicate that these recommendations are accurate and allow mental health professionals to precisely diagnose patients who do not accept illness, who are sensitive to worry and who are prone to affective disorders. Moreover, these specialists could supplement patient education programmes with psychotherapeutic content and interventions, designed according to the needs of specific patients. Unfortunately, despite the fact that the recommendations of the Polish Diabetes Association [52] also emphasize the significance of psychological interventions based on team care (including a psychologist), the presence of qualified health psychologists in Polish diabetes clinics is inadequate [53]. We believe that this is an area full of potential for development, and the current study can help by reintroducing the discussion concerning the situation of Polish diabetic patients in terms of clinical care.

The study includes several practical implications for specialists focusing on diabetes, but also in the area of research regarding the role of cognitive functions in adaptation to chronic diseases in general. Worry is a fairly common phenomenon in numerous chronic diseases. This fact was demonstrated in the meta-analysis conducted by Lebel, Mutsaers, Tomei at al. [54]. According to the authors, worry can occur in any chronic disease, although it affects various issues related to the course and characteristics of the condition, e.g. fear of recurrence (cancer), worrying about hypoglycaemia (diabetes), fear of pain (cardiac disease), etc. The above mentioned conclusions stem from the fact that so far, most studies on the phenomenon of worry in chronic diseases have been based on specific questionnaires, adapted to a specific problem and disease. Therefore, it is impossible to start a statistical discussion regarding the intensity and connotation of generalized worry in chronic diseases. The use of evaluation methods investigating the severity of generalized worry may contribute to the collection of data that in the future may support a cross-diagnostic analysis of the described problem. Our study shows the idea of using a metacognitive tool to collect data on gen-

eralized worry among people with diabetes. Furthermore, it may be interesting to compare the results concerning the phenomenon of worry and its relation to acceptance and depression in various chronic diseases.

The study has certain limitations, with the sample bias representing a major one, which should be addressed in the future. Patients involved in the study do not constitute a representative research group. Unfortunately, the research began in the pre-pandemic period, and the COVID-19 pandemic prevented the extension of the subject group in the same situational context. Moreover, the study did not include patients from the outpatient diabetes care or individuals who are not currently receiving clinical treatment. Future research involving a more diverse group of adults with T1DM may provide more data in this area. Thus, ongoing behavioural studies are necessary.

Conclusions

This study demonstrated that metacognitive processes, such as worry, contribute to depression among T1DM patients. In addition, the obtained data indicate that the age of patients may influence the general depressive mood, as well as the experienced somatic symptoms. Furthermore, a depressive mood occurring among patients with T1DM may persist due to the fact that the overly worried patients do not accept their own illness. One of the advantages of the present study was the use of research tools measuring the generalized worry, as well as the impact on the psychopathological components. The paper also emphasizes the importance of the presence of psychology specialists in T1DM patient's healthcare. Although the presented research has several limitations which need to be addressed in the future, we believe that it will contribute to the discussion regarding how metacognitive processes, such as worry, affect coping with a chronic disease.

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

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BRIEF REPORT

JMS Journal of Medical Science

Posttraumatic Stress Disorder symptoms in persons involved in road accidents and paramedics

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ABSTRACT

Aim. The aim of the study was to assess the risk of posttraumatic stress disorder (PTSD) among persons involved in road accidents and paramedics. Little is known about similarity or difference of PTSD symptoms between these two groups involved in accident in voluntary and involuntary way.
Material and Methods. Persons involved in road accidents (N = 78) and paramedics (N = 106) completed the Polish version of the Impact of Event Scale–Revised.
Results. The percentage of those who reported PTSD symptoms was similar and insignificant among persons involved in road accidents (56%) and among paramedics (45%). A significant difference ($p < 0,01$) was observed between these groups, however. The total PTSD, intrusions, and avoidance were higher for persons involved in road accidents.
Conclusions. Victims, perpetrators, and helpers in road accidents were at a similar risk of PTSD. Peritraumatic interventions are recommended for all these groups.

Introduction

Trauma has been defined as "a state of disruption caused by stressors severe enough to threaten life or make one believe that one is about to die" [1]. One of the possible consequences of a traumatic event, such as a natural disaster, a road accident, a war, a rape, etc., is posttraumatic stress disorder (PTSD) [2]. It is generally acknowledged that between 9% and 15% of individuals who are exposed to a traumatic event subsequently develop PTSD [1].

The ICD-10 criteria for PTSD (F43.1) are as follows [source: http://medical.cfoapublications.co.uk/12594]:

"A. The patient must have been exposed to a stressful event or situation of exceptionally threatening or catastrophic nature, which would be likely to cause pervasive distress in almost anyone.

B. There must be persistent remembering or 'reliving' of the stressor in intrusive 'flashbacks', vivid memories, or recurring dreams, or in experiencing distress when exposed to circumstances resembling or associated with the stressor.

C. The patient must exhibit an actual or preferred avoidance of circumstances resembling or associated with the stressor, which was not present before exposure to the stressor.

D. Either of the following must be present:
   - inability to recall, either partially or completely, some important aspects of the period of exposure to the stressor.
   - persistent symptoms of increased psychological sensitivity and arousal (not present before exposure to the stressor), shown by any two of the following:
     - » difficulty in falling or staying asleep
     - » irritability or outbursts of anger
     - » difficulty in concentrating
     - » hypervigilance
     - » exaggerated startle response.

E. Criteria B, C, and D must all be met within 6 months of the stressful event or the end of a period of stress (for some purposes, onset delayed more than 6 months may be included, but this should be clearly specified)"[3].

People exposed to PTSD include both victims of accidents [4,5] and first responders helping them, such as paramedics [6,7]. Being involved in a road accident is only a "potentially traumatic" event. Its consequence is the so-called peritraumatic response—the process of stress appearing as a result of the person realizing what has happened. This period is followed by a natural return to mental balance. Sometimes, however, the natural peritraumatic response extends and intensifies, leading to mental health disorders, such as PTSD. Accident perpetrators may suffer from PTSD as well [8].

Due to their occupation, paramedics providing assistance to persons involved in road accidents are also exposed to PTSD [9]. Additionally, paramedics may develop *Secondary Traumatic Stress Disorder* (STSD or Compassion Fatigue)—the consequences experienced as a result of heavy stress associated with helping traumatized people [10]. STSD affects people who are not directly exposed to a traumatic event but experience PTSD symptoms similar to those experienced by victims. *Disorders of Extreme Stress Not Otherwise Specified* (DESNOS or *Complex PTSD*) [11,12] is a set of symptoms resulting from prolonged and recurring trauma. This view is supported by data attesting to the multiple exposure of paramedics to traumatic events [13].

Being involved in a road/traffic accident is unintentional in the case of both the victim and the perpetrator, while the assistance provided by a paramedic suggests voluntary behavior. Due to the difference in intentions between these two groups, the following research hypothese was posed: There is a difference in the PTSD symptoms between paramedics and persons involved in road accidents.

Material and Methods

The participants in the anonymous and voluntary study were two groups: (1) persons involved in road accidents (N = 78) and (2) paramedics (N = 106). The former group consisted of 44 women and 34 men (M_{age} = 39.33, SD_{age} = 10.11); 72% of them were accident victims and 28% were perpetrators of accidents; 62% were drivers, 23% were passengers, 14% were pedestrians, and 1% were cyclists. As far as the type of accident is concerned, in 67% of cases it was a collision, 12% of accidents were knockdowns, 6% involved hitting an obstacle (a wall, a tree), and 15% involved other circumstances (a rollover, a skid, falling into a ditch). In the group of paramedics there were 43 women and 63 men (M_{age} = 29.51, SD_{age} = 5.64); all of them were professionally active and worked in units of the State Medical Rescue System in the Wielkopolskie Voivodeship (Province), Poland; their mean length of service was M = 7.35 years (SD = 5.98). When indicating traumatic events experienced during the week preceding the study, 49% paramedics reported a death, 13% reported an accident, 9% indicated aggressive behavior of patients or their families, and 4% reported a situation of threat to a patient's life.

Table 1. Descriptive statistics, reliability coefficients, and correlations for the variables measured using IES-R

	M	SD	Min.	Max.	α	PTSD	Intrusion	Hyperarousal
PTSD	37.56	11.39	0	85	.96	-		
Intrusion	15.56	9.53	0	36	.89	.94*		
Hyperarousal	10.31	6.32	0	24	.87	.92*	.88*	
Avoidance	14.27	7.37	0	29	.82	.82*	.61*	.62*

M = mean; SD = standard deviation; Min. - minimum value; Max- maximum value; α = Cronbach's alpha reliability statistic
*$p < .05$. **$p < .01$.

Table 2. Comparison of persons involved in road accidents and paramedics in the scope of PTSD

	Persons involved ***M (SD)*** **[Range]**	**Paramedics** ***M (SD)*** **[Range]**	t	df	p	d
PTSD (IES-r)	40.15 (19.62) [3-85]	31.99 (21.73) [0-78]	2.799	233	.006	0.39
Intrusion	15.56 (9.00) [1-36]	11.45 (8.16) [0-29]	3.521	233	<.001	0.48
Hyperarousal	10.32 (6.04) [0-29]	10.00 (7.56) [0-30]	0.326	233	.745	-
Avoidance	14.27 (6.83) [1-24]	10.54 (6.96) [0-10]	3.892	233	<.001	0.44

M = mean; SD = standard deviation; t – t-Student's test; df = degrees of freedom; d – Cohen's test

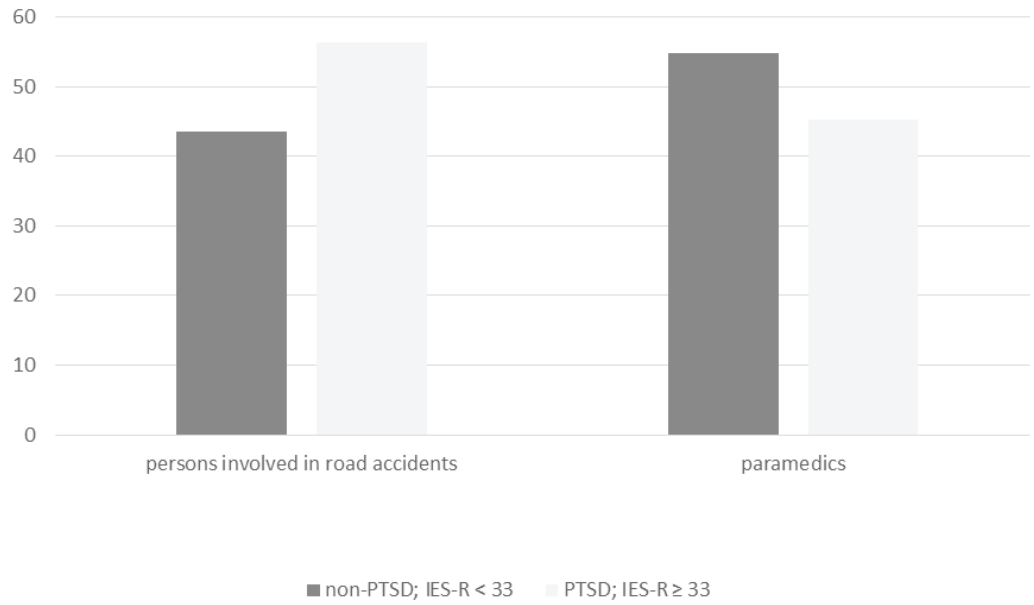

Figure 1. Percentages of participants with IES-R scores below and above 33 points among persons involved in road accidents and among paramedics

To assess the level of PTSD symptoms, we used the Polish version of the Impact Event Scale–Revised (IES-R) [14,15]. The IES-R consists of 22 items. Results include the total raw score and raw scores on three subscales: Intrusion (8 items; e.g., "I had dreams about it") , Avoidance (8 items; e.g., "I tried not to think about it"), and Hyperarousal (6 items; e.g., "I felt irritable and angry"). For each item, the respondent is asked to report the level of distress experienced in the past 7 days. The items are rated on a 5-point scale, from 0 = *not at all* to 4 = *extremely*. In general, the IES-R is not used to diagnose PTSD; however, cut-off scores for a preliminary diag-

nosis of PTSD have been cited in the literature (https://www.ptsd.va.gov/professional/assessment/adult-sr/ies-r.asp). The cut-off score of 33 points suggested a possible diagnosis of PTSD.

All procedures performed in study involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its further amendments or comparable ethical standards. This study was approved by the Bioethical Commission at Poznan University of Medical Science (Date: June 17, 2020, reference number 475/20). Written informed consent was obtained from all individual participants included in the study.

Results

A statistical analysis to test the hypothesis put forward was made in IBM SPSS Statistics, version 27. Key descriptive statistics were analysed with the use of the software, which made it possible to study the distributions of successive measured variables. Parametric tests were performed on all variables because skewness values did not exceed the conventional absolute value equals 2. The hypothesis was tested with the use of t-Student test. The significance level was adopted at the threshold of $p = 0.01$.

Basic descriptive statistics, reliability coefficients, and correlations for the study variables are presented in Table 1.

First, based on the cut-off score (IES-R ≥ 33 points), we identified the participants who might show PTSD symptoms (Figure 1). These participants accounted for 56% in the group of persons involved in accidents and 45% in the group of paramedics. The difference between these percentages was not statistically significant ($chi^2 = 2.610$, $df = 1$, $p = .128$).

The results of Student's *t*-test for the comparison of variables between independent groups are presented in Table 2.

The mean total IES-R score and the mean scores on the Intrusion and Avoidance scales are significantly higher in the group of persons involved in accidents than in the group of paramedics. The value of Cohen's *d* indicates a weak relationship between belonging to a particular group and PTSD dimensions.

Discussion

Persons involved in road accidents report a higher level of PTSD symptomatology than paramedics. This is the case for total subjective posttaumtic stress and intrusion (intrusive thoughts, nightmares, intrusive feelings and imagery, dissociative-like re-experiencing) and avoidance (numbing of responsiveness, avoidance of feelings, situations, and ideas). This result probably stems from the fact that for paramedics the occupational situation is intentional and voluntary, whereas for persons involved in accidents the situation is unintentional and involuntary. This perspective corresponds with the transactional understanding of stress as a loss/harm (one that has been suffered) or threat (anticipated loss) in persons involved in accidents, while in the case of paramedics the stressful situation is regarded as a challenge (anticipation of both losses and gains) [16]. The observed difference may also stem from the different organization of autobiographic memory. For paramedics, certain professional activities are routine; consequently, they have a general event scheme (referred to as script), which is based on the principle of generality and contains stable semantic knowledge. By contrast, in the memory of persons involved in accidents the accident is an episodic recollection, temporally organized and characterized by specific features, for which there is no scheme that could organize experience [17].

The observed difference can be also a consequence of presence of more women in group of persons involved in accident and age differentiation between groups. Women have a two to three times higher risk of developing PTSD compared to men [18]. As mentioned above, the group of persons involved in accidents was older than paramedics *ca.* 10 years. Data shows that middle-aged adults (ages 35-64) reported significantly higher the prevalence of past-year PTSD then young (ages 20-34) and experienced significantly more traumatic experiences [19].

In both groups the percentage of participants who report the presence of symptoms that indicate partial or subclinical PTSD is relatively high. Because in these groups there is a risk of PTSD development, interventions aimed at reducing the possible psychological costs are recommended. As part of peritraumatic prevention, individuals

who need professional help should be identified as quickly as possible through screening examinations by means of the IES-R and provided with professional assistance. Effective intervention should address both the emotional and cognitive spheres. It is recommended to apply, for instance, selected techniques of cognitive behavioral therapy from the prolonged exposure protocol [20], dedicated prevention programs for paramedics [21], or interventions for perpetrators of traffic accidents [22].

The fundamental limitation of the present study is the use of a self-report measure to assess a clinical disorder. IES-R score should be viewed in prognostic rather than diagnostic terms. This is because the measure yields the self-assessed level of posttraumatic stress symptoms, and it is only the use of in-depth clinical methods that allows for an objective diagnosis of PTSD. The next limitation is the modest sample. Another limitation is the heterogeneous group of persons involved in accidents, composed of both victims and perpetrators. It is true that victims and causers can experienced different affective states and consequences of involving in motor vehicle accidents e.g., anxiety [23] or self-blame [22]. But the presence of PTSD at these participants [8] makes that very often they are treated as non-identical but one group [4]. Probably it is justified when we realize that for victim and causer the car accident it is perceived inescapable, where they are tumbling out of control. This point of view corresponds with Type I traumatic events from Typology of Traumatic Events which refers to a single, discrete catastrophic event, such as a car accident or natural disaster. Although these events traumatize individuals, their responses are both attenuated or minimized by the balance of risk and protective factors [24].

In future studies, authors might want to include individual variables that may predispose both persons involved in road accidents and paramedics to coping with traumatic stress; they might also want to extend the scope of research to include employees of emergency services (e.g., firefighters or police officers) [25] or medical personnel (e.g., radiographers or surgical oncologists) [26]. The inclusion of these occupational groups would make it possible to broaden the research to cover the still insufficiently explored complex trauma or secondary trauma.

Conclusions

Conclusion: our results point toward the similar probability of the appearance of PTSD symptoms among road accident participants and paramedics. Although the declared presence of PTSD symptoms among paramedics is lower, it may be exacerbated by the work performed. Therefore, we recommend preventive programs in this professional group.

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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

The itinerary of circulating miRNAs – implications for cancer progression and diagnosis

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ABSTRACT

microRNAs (miRNAs) are non-coding RNAs that regulate gene expression and protect cells from foreign nucleic acids. miRNA is produced in the nucleus and processed in the cytoplasm. These small nucleic acid molecules are released from cells to the extracellular matrix (extracellular miRNA, ex-miRNA) and reach blood plasma (circulating miRNA). Circulating miRNA can also be detected in other biological fluids, such as saliva, cerebrospinal fluid or urine, and it is usually carried by proteins or extracellular vesicles. Argonaute-miRNA, or miRNA-lipoprotein complex, protect miRNA from being degraded. The entrance of extracellular miRNA into a target cell is mediated by endocytosis and membrane fusion of extracellular vesicles. Additionally, miRNA can also be delivered in high-density lipoproteins by means of interactions with scavenger receptors. miRNAs absorbed into a cell can act as tumour promoters (oncomirs), or suppressors by inhibiting the translation process of the target mRNAs, thus, affecting cells in the tumour microenvironment. miRNA can impact other cells by supporting tumour growth, promoting angiogenesis and modulating the immune system. Molecular high-throughput methods are employed to detect circulating miRNA, and a potentially helpful diagnostic test has been designed to characterise the cancer type. In this review, we aim to summarise the itinerary of miRNAs from a source cell to a target cell, as well as to show how this class of small nucleic acids participates in intercellular communication. Finally, we highlight examples of miRNAs usage as potential molecular markers and discuss treatment approaches in clinical trials.

Abbreviation list:
short interfering RNA – siRNA, microRNA – miRNA, precursor miRNA – pre-miRNA, RNA binding proteins – RBPs, tunnelling nanotubes – TnT, high-density lipoproteins – HDLs, argonaute 2 – Ago2, extracellular vesicles – EV, extracellular microRNA – ex-miRNA, toll-like receptors – TLR, heparan sulfate proteoglycans – HSPGs, heterogeneous ribonuclear protein E2 – hnRNP E2, non-coding RNA – ncRNA, tumour-necrosis factor-α (TNF-α), tumour microenvironment – TME, cancer-associated fibroblast – CAF, vascular endothelial cadherin – VE-cadherin, human umbilical vein endothelial cells monolayer – HUVEC, polyethyleneimine -PEI.

1. Synthesis and release of miRNA molecules into the intercellular space

1.1. miRNA synthesis

Different types of small RNAs, including miRNAs, tRNAs, rRNAs and yRNAs (YRNAs – small non-coding RNAs essential for the initiation of chromosomal DNA replication), have been detected in the extracellular matrix and circulation [1]. In the last decades, small RNAs have gained new significance as regulators of eukaryotic genomes. Mature miRNA is a single-stranded RNA derived from a double-stranded precursor with 20-22 base pairs in length. It regulates gene expression and defends cells against invasive extracellular nucleic acids, and recent research proves its diversity in terms of biogenesis pathways and regulatory mechanisms [2]. Furthermore, miRNAs are the most broadly distributed in both phylogenetic and physiological terms, therefore, we will focus on these molecules, as since they are secreted and absorbed by cells, they could therefore be considered as signalling molecules.

The biogenesis of miRNA constitutes a multistage process in which both nuclear and cytoplasmic enzymes have been addressed in a number of reviews, e.g. Bartel (Figure 1) [2]. According to the studies, miRNAs could regulate gene expression in the cell, or could be exported to the extracellular matrix by means of extracellular vesicles [1].

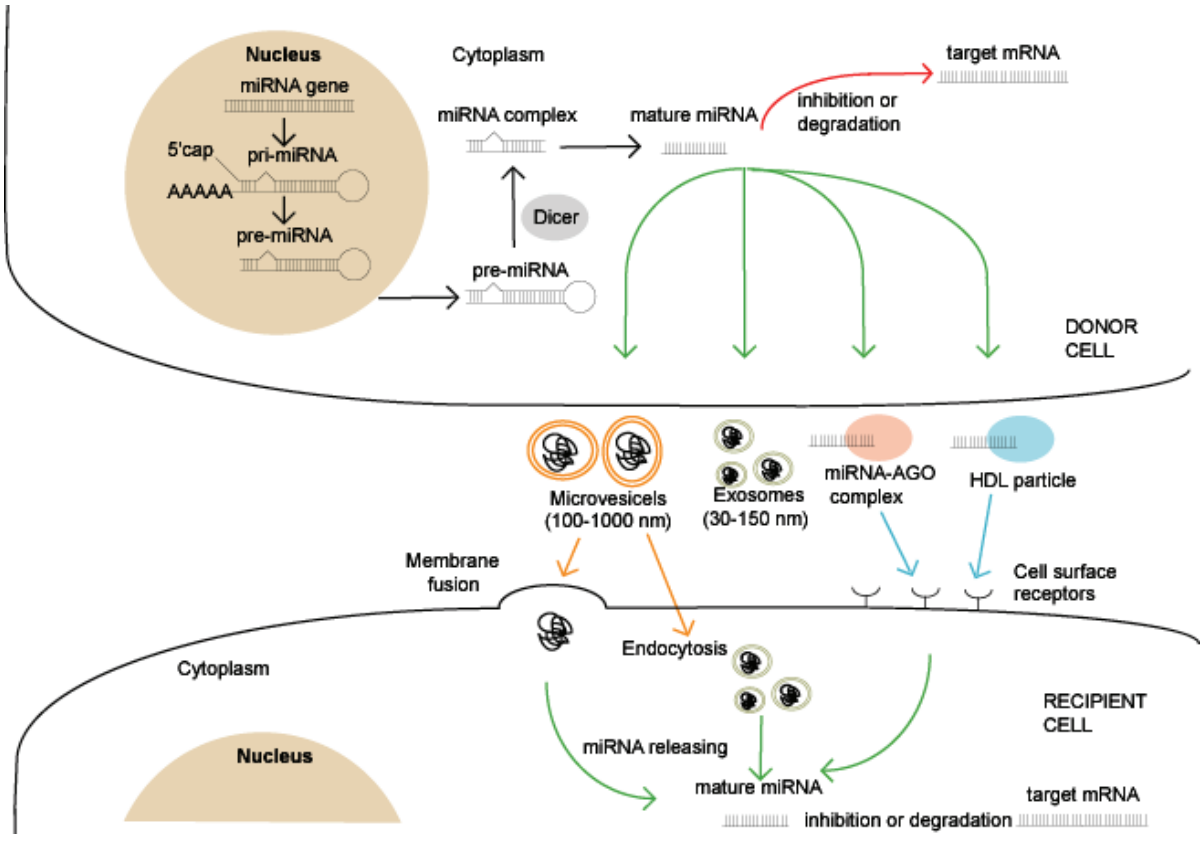

Figure 1. miRNA biogenesis, secretion and uptake

The miRNA genes are transcribed in the nucleus to produce primary miRNA (pri-miRNA). Subsequently, pri-miRNA is further converted to the precursor miRNA (pre-miRNA) and transported to the cytoplasm. In the cytoplasm, an enzyme Dicer cleaves pre-miRNA into miRNA duplex, which eventually releases mature miRNA. In turn, mature miRNA mainly targets cytoplasmic mRNA, resulting in the translation inhibition or mRNA degradation. Additionally, miRNA can also be secreted into the extracellular environment, which allows it to reach distant cells.

Extracellular miRNA may be surrounded by a vesicle-associated membrane. The transporting vesicles of a normal cell include exosomes (30-150 nm) and microvesicles (100-1000 nm). However, the extracellular miRNA may be also non-vesicle associated, when carried by ribonucleoproteins, mostly by Argonaute 2 (Ago2), as well as transported with high density lipoproteins (HDL).

Once miRNA reaches the recipient cell, the uptake commences. Exosomes and microvesicles can be absorbed by recipient cells by endocytosis or by direct fusion with the plasma membrane. HDL-associated miRNAs and miRNA-AGO2 complexes are taken up by the recipient cells through binding to the specific receptors present in the cellular membrane.

Following the release in the recipient cells, miRNAs control protein synthesis, mainly by means of targeting mRNA, which further leads to its inhibition or degradation.

1.2. miRNA sorting into exosomes

Exosomes are 30 to 100 nm-sized type of extracellular vesicles secreted by most cell types into the extracellular space and were broadly characterised in *Minimal information for studies of extracellular vesicles* [3]. In fact, as carriers of different biomolecules, including proteins, lipids or nucleic acids, exosomes play an essential role in physiological and pathological states [4]. They participate in cell-to-cell communication and act as mediators, modulating the activity of other cells, with exosome content being transported not only into the surrounding cells, but also to more distant tissues [1]. Moreover, they are capable of triggering a systemic response. However, the mechanisms of information exchange between cells are extensive and not fully understood.

In terms of the exosomal resident transcripts, particular attention has been given to miRNAs, due to their high conservation across species, involvement in gene expression regulation and the role of signalling molecules in metastatic tumour cell growth. Unfortunately, the molecular mechanisms controlling the specific loading of miRNAs into exosomes remain poorly understood. Both selecting and non-selecting mechanisms have been described in this context, and it is suggested that several loading mechanisms may govern exosome sorting of specific subsets of miRNAs [5, 6]. In fact, on the basis of the current research, several pathways have been described which impact sorting of miRNAs into exosomes, i.e. 1) the neutral sphingomyelinase 2 (nSMase2) membrane protein-dependent pathway; 2) the loading of miRNAs controlled by hnRNPA2B1 SUMOylation, 3) the 3'-end of the miRNA sequence-dependent pathway; 4) the miRNA induced silencing complex (miRISC)-related pathway, where Argonaute 2 (Ago2) is reportededly involved in the sorting of such genes as let-7a, miR-100, and miR-320a; 5) other RNA-binding proteins related pathways since the conducted studies proved that specific proteins might govern miRNA sorting by means of recognising and binding to specific RNA sequences called the EXO motif [7]. Moreover, research conducted to date has demonstrated that miRNA are selectively packed into exosomes by a mechanism dependent on RNA binding proteins (RBPs) [7], and RBPs, in turn, form complexes with RNAs and transport them into exosomes during biosynthesis [8].

There is evidence that short sequence motifs (EXOmotifs) are overrepresented in miRNAs and connected with the process of sorting the miRNAs into exosomes, e.g. hnRNPA2B1 complex binds and loads specifically about 30 exosomal miRNAs through the recognition of GGAG motif (e.g. identified in the T cells) [9]. Furthermore, it has been reported that some miRNAs proved to promote cancer metastasis and are selectively sorted into exosomes, such as miR-122 containing complex, bipartite motifs (UGGA at the 5' end, UUU at the 3' end) [6]. Additionally, identifying the GCAG motif in the miR-1246 sequences further supports the concept that RNA sequence motifs are a crucial factor responsible for the selective cellular miRNA sorting into exosomes [10].

2. Close and distant miRNA peregrinations outside the cell

Extracellular RNA is found in biological fluids in large quantities and is detected in saliva, blood, cerebrospinal fluid, breast milk, semen, and urine [11]. RNA present in the extracellular fluids originates from endogenous and exogenous sources. miRNA can be expelled from cells due to cell death, in the course of apoptosis or necrosis [12]. Moreover, RNA may also be released as a paracrine signal for communication with other cells. The exogenous extracellular RNA might be derived from another organism (foreign RNA) and can be exchanged between different kingdoms in the horizontal transfer process. In fact, horizontal transfer has been observed between RNA viruses and eukaryotes, plants and viruses, plants and mammals or other animals [13, 14].

In general, the concentration of miRNA in the extracellular space is much smaller than in cells, due to RNA-degrading enzymes in biofluids [15]. Blood cells constitute a significant contributor to the circulating miRNA [16]. However, extracellular miRNA profiling is affected by gender, age, metabolism, diet, physical exercise and general health condition. miRNAs are transported from cell-to-cell in membrane-derived vesicles, high-density lipoprotein particles (HDLs), apoptotic bodies and a short distance through tunnelling nanotubes (TnT) [17]. In addition, miRNA is protected in the extracellular space from ribonucleases (RNases) and other degrading factors by being transported with proteins. 500 known transporting proteins may have a specific affinity, forming stable ribonucleoprotein complexes with RNA and it is assumed that more than 90% of circulating miRNAs in plasma are associated with proteins [18]. The most abundant form of extracellular RNA (ex-miRNA) in the blood is bound with Ago2, suggesting that the RNA protection mechanism may be the most effective. Another ribonucleoprotein involved in the transport, stabilisation and protection of miRNA is nucleophosmin 1 (NPM1). Primarily, NMP1 has been known as a nucleolus protein, participating in ribosomal RNA processing. According to the studies by Kai Wang et al., NPM1 participates in the exportation and ex-miRNA protection [19], but it may also be transported with high-density lipoproteins [20].

miRNA can be transported between cells through direct connections without entering the extracellular space. One of the direct connections is referred to as TnT and connect cells and communicate by electrical signals, exchange proteins, nucleic acids and organelles [21]. Another way for miRNA to be transported between cells is a gap junction, which directly connects two cells by a channel consisting of connexins. Although gap junctions are used predominantly to transport small molecules, such as water or ions, they also transport RNA, proteins and other molecules [22].

In healthy individuals, the amount of vesicle-associated RNA is minimal, since most extracellular RNA is protein-associated; conversely, it increases in pathological states. Interestingly, breast cancer patients present significantly increased levels of vesicle-associated exosomal RNA, which may indicate the degree of disease severity [23]. Thus, the amount of extracellular RNA, its type and its method of transport are affected by various factors, and, due to that, they differ in certain organisms and tissues, which should be taken into consideration in its analysis.

Approximately $1–3\times10^{12}$ exosomes could be derived from 1 ml of the human serum, and these exosomes contain 0.5 – 2.5 ng of RNA [24], and miRNAs levels for various cell types are estimated in the range of tens to 120,000 copies per cell [25]. The variety of miRNAs present in extracellular vesicles could become a diagnostic marker for breast cancer. EVs released from hepatocytes could indicate liver insufficiency, whereas exosomes from the placenta might become a diagnostic biomarker of the foetal disease [26]. According to the studies, specific miRNA is only associated with EVs, such as let-7a [27].

HDL had become the focus of new studies which suggest that HDL is a miRNA transporter and a protector from RNases in the circulatory system. Nevertheless, the process of releasing miRNA associated with HDL and the role of HDL in gene modification remains unknown. An investigation of miRNA-HDL complexes by Wagner et al. presents that miR-223, miR-92a, and miR-126 are more likely to be associated with HDL. However, the highest ratio of miRNA-HDLA complex to the entire circulating miRNA was below 10%, indicating that HDL cannot be a significant transporter of miRNA. The same study referred to a specific miRNA associated with the low-density lipopro-

tein (LDL), miR-155, which resulted in atherosclerosis in more vulnerable organisms [28].

It has been suggested that circulating miRNA-protein complexes are able to participate in paracrine functions, and that miRNA encapsulated in exosomes might play a role in intercellular communication [18]. Furthermore, investigation of miRNA concentration kinetics in the blood is required, particularly for miRNA associated with exosomes. These particles contributing to intercellular communication could be molecular capsules involved in drug delivery to cells [18].

3. Transfer from the circulation into the cells

3.1. RNA entrance into target cells and the impact on gene expression

Several mechanisms allow for the entrance of RNA into target cells, thus affecting gene expression. One mechanism of RNA entering into cells is internalisation mediated via exosomes. In fact, they play a role in the communication of neoplastic cells and deliver mRNAs and miRNAs into target cells [29]. Zhang et al. demonstrated that the miR-150 packaged into exosomes entered Human Microvascular Endothelial Cells (HMEC-1 cells) [30]. The binding of exosomes onto target cells can be mediated through receptors present on the cell surface, with MHC I, MHC II, tetraspanins, and transferrin being the receptors involved in exosome transportation [31]. The exosome membrane surface, especially proteins, could be investigated as potential mediators of exosome docking on the target cell membrane [29]. Subsequently fusion of exosomes with the cell membrane occurs, which could be limited by the low pH of the tumour microenvironment, since the optimal pH of the membrane internalisation is about 5.0. Therefore, this pH is optimal to achieve the highest fluidity of membranes and fusion potential [32]. Furthermore, endocytosis, including phagocytosis, is also an established internalisation mechanism [31]. Heparan sulfate proteoglycans (HSPGs) were described as mediators in exosome internalisation with destination cells, in particular in exosomes released by cancer cells. Moreover, it has been demonstrated that HSPGs play a significant role in the uptake of exosomes. This phenomenon could be important for the development of exosomes delivering RNA therapeutics into cancer cells.

miRNAs, present in the circulatory system and extracellular fluid compartments, can affect cells located far away from the donor cell, and as a result, they can alter cell gene expression [33]. The miRNA activity could be referred to as "hormone-like", since this molecule requires interaction with a receptor situated within the cell, or on the cell membrane. The possible mechanism of hormone-like activity of miRNA involves a protein receptor for miRNA (miRceptor) and a potential miRNA-protein interaction which was first observed in 2010 [34]. Direct miRNA-protein interplay occurs in chronic myelogenous leukaemia cells between miR-328 and the heterogeneous ribonuclear protein E2 (hnRNP E2). hnRNP E2 inhibits CCAAT/enhancer-binding protein α (CEBP-α), leading to an inhibition in granulocytic differentiation. The best-known mechanism of controlling gene expression by miRNA is the repression of translation. It is performed by a pairing of six bases in 5'-end located in miRNA seed region with the 3'UTR sequence on the target mRNAs and, as a result, inhibiting the translation or, more radically, initiating the degradation of mRNA [2].

The aforementioned mechanisms of gene regulation are also mediated by incoming miRNA in the target cells. By means of the transport through EVs, the expression of miR-409 in stromal fibroblasts can affect other cells and promote tumorigenesis via repression of tumour suppressors, such as RAS suppressor 1 (RSU1), as it was demonstrated in prostate fibroblasts [35]. On the other hand, miRNA, such as miR-135a, is also involved in tumour growth repression, where it suppresses the invasiveness of prostate cancer cells by impacting ROCK1 and ROCK2 [36].

3.2. The role of imported miRNAs in the target cells – preparation for cancer progression

In order to better understand the role of miRNA on cells, one should look at the immediate environment of the tumour. The tumour microenvironment (TME) is defined as the area enclosing the tumour which contains cancer as well as stromal cells, fibroblast, and immune cells. The TME is rich in signalling molecules, blood vessels, and the extracellular matrix (ECM) [37]. The TME creates optimal conditions for tumour development,

growth and metastasis, supplies nutrients and provides a physical scaffold for cells [37]. miRNAs, secreted by the tumour cells, can impact other cells in the TME on several levels. Finally, similarly to hormones, miRNAs can be transported through the bloodstream to modify a healthy microenvironment, making it suitable for metastasis. Recent studies have indicated that miRNAs play a significant role in metastasis [38]. In this paper we report the representative examples of how miRNA can modify a local and distant cell microenvironment, so as to promote tumour growth and enhance metastasis formation.

3.2.1. Modulation of the vascular permeability and angiogenesis

The ability of cancer to form metastasis and invade distant organs requires miRNAs to facilitate this process by promoting vascular permeability and angiogenesis. miR-105 was reported to destroy the vascular endothelial barrier and to promote metastasis by targeting the tight junction protein ZO-1. As a result of the destruction of tight junctions, the integrity of the barrier is compromised, increasing vascular permeability and leading to metastatic progression [39]. Other studies have demonstrated that over-expression of miR-939 targeting vascular endothelial cadherin (VE-cadherin) results in an increased permeability of human umbilical vein endothelial cells (HUVEC) [40]. Finally, miRNA is involved in forming new vessels, and although miRNA alone is not sufficient to induce metastasis, they greatly contribute to the process.

3.2.2. Immune modulation

Tumour cells secrete extracellular vesicles transporting miRNA, which are taken up by surrounding cells in the tumour microenvironment and by cells in distant organs [41]. It is worth noting that tumour extracellular vesicles can promote cancer angiogenesis, invasion and metastasis. Immune cells of myeloid and lymphoid origin are part of the tumour microenvironment, with myeloid cells being widely present in tissues. In fact, they comprise most immune cells and play an essential role in immune reactions and tissue remodelling. Different subsets of mature myeloid cells, such as monocytes, macrophages, dendritic cells and granulocytes take up EV and acquire a pro-tumorigenic phenotype. In the course of neoplasm formation, the differentiation of myeloid cells and their functions are impaired, resulting in tumour promotion. A high capacity of myeloid cells to EV uptake from circulation enables the formation of pre-metastatic niches [42]. The exposure of myeloid cells to tumour EV could inhibit the anti-tumour function of T cells and natural killer cells and promote tumour progression. Hence, immune cells establish a pro-inflammatory environment, which stimulates tumour growth and partially suppresses the anti-cancer immune response [43]. Moreover, lung cancer cells secrete miR-21 and miR-29a, which bind and activate toll-like receptors (TLR) 7 and 8, inducing the secretion of pro-inflammatory cytokines, such as IL-6 and TNF-α which facilitate tumour growth and metastasis formation [44]. Although immense data indicate the involvement of miRNA in immunomodulation of tumorigenesis, the participation of circulating miRNA in the process has not been well described. Nevertheless, it has been reported that miR-195 and miR-497 inhibit CD274 expression, affecting the immune response. Other studies have shown that overexpression of miR-203 in pancreatic adenocarcinoma leads to downregulation of TLR4 on pancreatic dendritic cells, inducing immune tolerance [45]. Similarly, miR-212-3p inhibits regulatory factor X-associated protein, i.e. an important transcription factor for major histocompatibility complex (MHC) II, which results in a decreased MHC II expression and immune tolerance of dendritic cells in pancreatic carcinoma [46].

3.2.3. Drug resistance

miRNAs contribute to the drug resistance of tumour cells by means of targeting genes related to cell proliferation, cell cycle, and apoptosis. Moreover, drug-resistant tumour cells can spread resistance to other non-resistant cells, which leads to treatment failure [47]. In the MCF-7 breast cancer cell line, the altered expression of miRNA-21 affects the susceptibility to doxorubicin used to treat breast cancer. Additionally, other studies have demonstrated that upregulated expression of miR-106a contributes to cisplatin resistance in non-small cell lung carcinoma [48]. Similarly, miR-15b increases the resistance of lung adenocarcinoma cells to cisplatin by suppressing phosphatidylethanolamine-binding protein 4 [49]. The miR-23a overexpression in colon

cancer cells is reported to lead to resistance to 5-fluorouracil, with the mechanism of miR-23a-induced resistance decreasing the level of apoptosis-activating factor-1 in the colorectal cancer cells [50]. Furthermore, exosomal short-distance transfer of miRNAs may also lead to the development of chemoresistance. Tumour-associated macrophages receive miR-1246 abundant exosomes derived from paclitaxel-resistant epithelial ovarian carcinoma cells, and miR-1246 further promotes paclitaxel resistance. In contrast, epithelial ovarian carcinoma cells receive miR-21 abundant exosomes from cancer-associated adipocytes and fibroblasts, resulting in paclitaxel resistance. Moreover, in hypoxic conditions, miR-223 enriched tumour-associated macrophage-derived exosomes promote cisplatin resistance in epithelial ovarian carcinoma cells via the PTEN-PI3K/AKT pathway [51]. Interestingly, miRNAs may also be associated with melanoma resistant to treatment with immune checkpoint inhibitors (ICIs) [52].

Recent studies have suggested that cancer-derived exo-miRs are released in a different pattern, which is assumed to display multi-drug resistance through the mechanisms described above [53]. As described in a recent review, secreted exo-miRs prepare the cell microenvironment for cancer evolution, therefore, chemotherapy resistance affects all types of tumour cells [53]. On the other hand, microenvironment cells (such as macrophages and fibroblasts) are considered to transmit multi-drug resistance to sensitive neighbouring cells. In all of the above-mentioned scenarios, exo-miRs play a major role in cancer drug resistance.

4. Diagnostic significance of circulating miRNAs.

Shortly after the discovery of circulating miRNA, these molecules have been observed to be potential diagnostic and prognostic biomarkers in cancer. There are at least three main objectives in the use of circulating miRNA as cancer markers: early diagnosis markers (predictive biomarkers), molecular classifiers, as well as relapse monitoring markers (prognostic biomarkers) [54]. The predictive biomarkers could be helpful in the regular monitoring of a high-risk group of patients. There are numerous examples of studies aiming to elaborate on predictive biomarkers [55]. For instance, the MiR-17-92 is one of the oncogenic miRNA clusters involved in colorectal carcinogenesis, and levels of MiR-17, miR-18a, miR19a, miR-20a, miR-19b and miR-92a – members of this cluster increase in colorectal cancer plasma samples [55]. Hence, it is of importance, as early detection of colorectal cancer, markedly improves the prognosis. Other studies have also associated the expression of miR-17 also with cervical cancer [56]. The most frequent neoplasm in women is breast cancer, which is characterised by heterogeneity and variable subtypes differing in grade and malignancy, which in consequence, necessitates a diversified therapeutic approach. In order to distinguish breast cancer types, reliable molecular classifiers markers have been investigated [57]. As a result, it was demonstrated that the miRNAs expression profile could distinguish the triple-negative breast cancer from other molecular breast cancer subtypes [58, 59]. The relapse prognostic biomarkers could be a form of post-surgery or a radiotherapy monitoring marker. In post-surgical plasma samples, the tests showed good performance with regard to monitoring disease relapse.

On the basis of screening cohorts comprising lung cancer patients, circulating miRNA pre-test were designed by selecting miRNA biomarkers — a non-commercial experimental miRNA signature classifier test based on a microfluidics card containing the 24 miRNAs. Although the miR-Test uses 13-miRNA types, such experimental tests resulted in a fourfold to fivefold reductions in the low-dose computed tomography false-positive rate [60, 61]. ThyraMIR classifies the gene expressions of ten microRNAs, and it allows physicians to identify thyroid cancer with a single test. According to the supplier, ThyraMIR and ThyGenX present a specificity of 85% and sensitivity of 89%. ThyraMIR uses the expression levels of 10 microRNAs, whereas ThyGenX aims at mutations or genetic alterations associated with thyroid cancer. In combination, the two tests are used to produce a positive or negative result from the routine fine-needle aspiration procedures [62].

The significance of circulating miRNA in diagnostic tests has been developing dynamically in the breast cancer area. Researchers discovered that the concentration of miRNA-29b-2 and miR-

NA-155 was higher in blood serum in samples from patients who suffer from breast cancer compared to non-breast cancer patients. Therefore, these findings could suggest that an increase in the concentration of certain molecules of miRNA in the blood serum may result from oncogene activation. Conversely, the amount of other types of miRNA decreases in the aftermath of a suppressor gene turning off. Taking the abovementioned facts into account, diagnostic kits should be developed which would reliably exclude or confirm the diagnosis of breast cancer or enable the development of cancer screening with miRNA.

In the past, research regarding the efficacy of miRNA panels in the detection of colorectal cancer was conducted. Researchers investigated examined a panel of 8 miRNAs, which distinguish patients with polyps from patients without them (miRNA-532-3p, miRNA-331, miRNA-195, miRNA-17, miRNA-142-3p, miRNA-15b, miRNA-532, and miRNA-652) and a panel of 3 miRNAs, which distinguish patients with stage IV colorectal cancer from patients without colorectal cancer (miRNA-431, miRNA-15b, and miRNA-139-3p). The differentiation between patients with and without cancer by the panels demonstrated high accuracy. The sensitivity of the test, both in the group of patients with colorectal adenomas and the control group, amounted to 88%, while the specificity reached 64%. In addition, the comparison between stage IV colorectal cancer patients and the control group was 93% and 74%, respectively [63].

Several similar studies have shown that breast cancer [56], prostate [64], colorectal [65], gastric and others [65] are all candidates for 'potential' miRNA biomarkers This, in turn, raises the question as to why the considerable data on the potential biomarkers have not been efficiently transformed into clinically useful diagnostic tests [66]. The information on the website *https://clinicaltrials.gov/* concerning recent ongoing clinical trials involving miRNA biomarkers, comprises one completed study '*Circulating miRNAs as Biomarkers of Hormone Sensitivity in Breast Cancer* in phase IV, no studies in phase III, one study in phase II and one study in phase I. In order to be able to make a correct clinical diagnosis of a patient's sample, the most critical evaluation criteria for biomarkers are high sensitivity and specificity [67]. miRNA and expression markers are usually determined on a large cohort, and as such display some bias and rarely reach sufficient specificity and reproducibility in a particular patient sample. This is problematic for miRNAs, which are deregulated due to primary genetic or epigenetic alterations [68]. Furthermore, a number of uncontrollable factors and stochastic fluctuations hinder the estimation of miRNA level in circulation. Recent works suggest genetically programmed-in fluctuations within miRNA pools, possibly driving the formation of adaptive phenotypes [69].

This poses the question, whether there are any advantages to using miRNA markers miRNA is relatively stable in ex vivo blood samples and over 200 types detectable in blood serum by the most sensitive method. Therefore, this broad spectrum of molecules enables the recruitment of several reliable sequences to increase the specificity of a potential test [67]. Circulating miRNAs for diagnostic tests are collected as a liquid biopsy from plasma or serum [70]; nevertheless, easily accessible plasma and serum vary, and so different results are obtained [66]. Serum is considered a better option, since it is less likely to contain products of haemolysis. A decreased circulating miRNA level compared with the cytoplasmic miRNA concentration requires the use of particular methods to detect it [71]. The two most common methods to detect miRNA in serum are microarrays and quantitative reverse transcriptase real-time PCR (RT-qPCR) [72].

The disadvantages of miRNA markers might seemingly outweigh the advantages. In fact, miRNAs indicates altered expression patterns in the same type of cancer assayed by different studies, and miRNA levels are dependent on age, gender, ethnicity, lifestyle, pre-treatment, history of diseases. Additionally, in terms of detection results, they can be affected by the measurement principle, the method used and the instrument and also haemolysis should be strictly controlled in plasma samples [67]. In a relative quantification of circulating miRNA, the lack of a normaliser is the most significant obstacle. An additional challenge is the fact that miRNA is predominantly secreted by healthy cells; hence, a multi-parameter assay instead of a single marker has been proposed to allow miRNA biomarker testing. However, it is has recently been suggested that in order to develop a specific test the absolute level of miRNA should be considered as more reliable [73].

5. Application of miRNA as drugs and vaccines

miRNA, as an essential tumour suppressing factor, could be used to inhibit oncogenesis. Several anti-micro-RNA therapies have already been described by Miroshnichenko et al. [74]. Here we present several examples of promising drugs based on RNA oligonucleotides which have already been in clinical trials.

The gene encoding miR-34 is regulated by p53 and has been observed to be decreased in several types of cancer, including prostate cancer, pancreas cancer, breast cancer and many others. Nanoparticles containing miR-34 inhibit the development of a tumour, metastasis and stimulate apoptosis. MRX34 is a form of nanoparticles containing RNA mimic tested in the first phase of the study where the drug consisted of 23-nucleotides closed in a liposomal nanoparticle [75]. *MYC*, *MET*, *BCL2*, and *WNT 1/3* are examples of inhibited oncogenes by MRX34 (93). A clinical trial has shown plenty of side effects occurring as a result of miR-34 therapy including immune responses, which probably resulted from suppressed immune-related genes by miR-34 [75]. Thus, MRX34 did not move to the second phase of trials due to high toxicity [76].

Imetelstat (GRN163L) is an oligonucleotide, a second phase trial drug, which acts as RNA's telomerase inhibitor. GRN163L exhibits antiproliferative and cytotoxic effects. The treatment of haematological cancers and solid tumours affects the delay in megakaryopoiesis [77]. The research presents possible action on reducing malicious hematopoietic stem cells and hematopoietic progenitor cells in myelofibrosis. Nevertheless, thrombocytopenia constitutes the toxicity factor limiting the drug's dosage [78].

An exciting novelty among miRNA's drugs is RGLS5579. The oligonucleotide inhibits miR-10b, whereas the overexpression of miR-10b occurs in colorectal and breast cancer, where it promotes metastasis [79]. The drug could be administered to patients diagnosed with glioblastoma multiforme, which is the most aggressive type of cancer of the brain [76].

"TargomiR" technology is based on targeted minicells containing miRNA mimic [80]. The first drug using this novelty technique is MesomiR-1. The first phase of clinical trials was completed, as a result of which MesomiR-1 based on miR-16 was most likely to suppresses tumours on the scale of cancer types [79]. The research was conducted on patients with malignant pleural mesothelioma and non-small-cell lung carcinoma. The drug was considered safe due to benign side effects [81].

The role of miR-155 is an oncomir regulating pathways in immune cells. Cobomarsen (MRG-106) is introduced as a synthetic anti-miR-155 oligonucleotide in second phase trials. The drug was administered to patients with haematological malignancies [82]. Cobomirsen decreases cellular proliferation and generates apoptosis in *mycosis fungoides*. MGR-106 is currently being tested for safe use and tolerability.

siRNA drugs, based on RNA interference, degrade mRNA in a long-lasting period. It is essential to deliver the drug in lipid or N-acetylgalactosamine (GalNAc)-conjugated nanoparticles to enable transport of siRNA into hepatocytes. [83]. Gene knockdown effects of double-stranded siRNA therapeutics were used in several clinical trials. APN401, EphA2 siRNA, ALN-VSP02, CALAA-01 and DCR-MYC are examples of drugs remaining in the first phase. siG12D-LODER and TKM-080301 were classified for the second phase trials [84].

The delivery of EVs from the tumour into the destination cells could be facilitated by means of polyethyleneimine (PEI). PEI is the synthetic polymer of different molecular weights generated by aziridine monomers. The modification of EVs with PEI/siRNA complexes significantly increases RNA molecules' uptake carried by exosomes. This uptake may improve the uptake of exosome-mediated drug transfer against cancer, although it was mainly investigated *in vitro* [85]. Several RNA-based vaccines contain genes situated in a bacterial plasmid. The majority of vaccines do not have preventive activity, although they are based on the stimulation of cell responses. Dendritic cells are used to deliver mRNA to the cells. Dendritic cells with antigens related to melanoma combined with monoclonal antibodies against CTL antigen four results in a decrease in tumour size. Another solution of mRNA application includes a direct injection into the secondary lymphoid tissue, intranasal, intratumoral, intradermal, systemic administration using lipid-based carriers [86]. The table presenting clinical trials with mRNA vaccines has been published by Pardi et al.[86].

5. Conclusions

It is difficult to deny the effect of miRNAs secreted by cancer cells on other body cells. However, despite extensive research, there are still issues with regard to the understanding the selection of miRNAs packed into EVs. The mechanisms of the selective uptake of EVs by cells from extracellular space remains obscure. Nevertheless, the phenomenon of gene expression regulation by incoming small amounts of miRNA in recipient cells requires further research. miRNA has excellent potential for future use as a biomarker, and more efforts should be made to reveal its mechanisms in order to develop new diagnostic and treatment methods.

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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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Ethical issues on artificial intelligence in radiology: how is it reported in research articles? The current state and future directions

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ABSTRACT

Background. This paper evaluates the status of reporting information related to the usage and ethical issues of artificial intelligence (AI) procedures in clinical trial (CT) papers focussed on radiology issues as well as other (non-trial) original radiology articles (OA).

Material and Methods. The evaluation was performed by three independent observers who were, respectively physicist, physician and computer scientist. The analysis was performed for two groups of publications, i.e., for CT and OA. Each group included 30 papers published from 2018 to 2020, published before guidelines proposed by Liu et al. (Nat Med. 2020; 26:1364-1374). The set of items used to catalogue and to verify the ethical status of the AI reporting was developed using the above-mentioned guidelines.

Results. Most of the reviewed studies, clearly stated their use of AI methods and more importantly, almost all tried to address relevant clinical questions. Although in most of the studies, patient inclusion and exclusion criteria were presented, the widespread lack of rigorous descriptions of the study design apart from a detailed explanation of the AI approach itself is noticeable. Few of the chosen studies provided information about anonymization of data and the process of secure data sharing. Only a few studies explore the patterns of incorrect predictions by the proposed AI tools and their possible reasons.

Conclusion. Results of review support idea of implementation of uniform guidelines for designing and reporting studies with use of AI tools. Such guidelines help to design robust, transparent and reproducible tools for use in real life.

Introduction

Advances in radiology directly correlate with developments in imaging technology [1]. Developing new imaging modalities or increasing the efficacy of already implemented solutions improves the decision-making process in routine work of radiologists making their analysis more accurate. To implement new hardware solutions with dedicated software from factory to clinic, an appropriate certificate and regulation (e.g., Conformité Européenne, Food and Drug Administration, EU Medical Device Regulation) needs to be obtained and, then, the usefulness of solutions needs to be carefully evaluated in specific areas of usage. After this process is over, the use of these tools is clearly defined and established in routine work. The challenge starts when the machine ceases to be a tool in the hands of radiologists and becomes their advisor, e.g., decision support systems based on artificial intelligence (AI).

AI describes a range of techniques that allow computers to perform tasks that require human reasoning and problem-solving skills [2]. AI is encapsulated in software for which advanced mathematical algorithms (e.g., machine learning) are implemented to automate work or support human decisions [3,4]. AI is not a new concept in radiology. Over the last 10 years (from 2010 to 2020), over 6,000 original papers describing the implementation and use of AI methods in radiology have been published (source: authors' search with the PubMed engine). However, for radiologists, AI is a new tool that not only gives the radiologist the content for interpretation but also tries to interpret this content for them. This fact revolutionized common thinking about radiology tools and forced the radiologist community to redefine tools used in routine work, especially in legal and ethical terms. Indeed, in the last three years (from 2017 to 2020) over 100 statements, editorials, review and commentary articles have been published about ethical aspects of AI usage in radiology (source: authors' search with the PubMed engine). These papers focused on fundamental ethical aspects of diagnostics, as well as ethical issues connected to every step of the diagnostic process supported by AI. Neri *et al*. [5] emphasize that it is the radiologist who is responsible for diagnosis, not the AI tool, designed to support it. Patients should always sign informed consent for their data to be used in this non-conventional way. The radiologist should know how to use AI tools. AI operating patterns should be transparent and as clear as possible and, finally, when using AI tools radiologists need to take responsibility for the accuracy of the AI suggestion as it may bias their final diagnosis. The European and North American multi-society statement [6] is one of the essential papers that describe in detail every step of the diagnostic process supported by AI. This multi-institutional report identified three main areas of the process that require new regulations. These are: data processing, transparency of algorithms and trained models and the relationship between patients and radiologists. While the statement answers "*why*" it is needed, Brady and Neri [7] tried to answer "*how*" to do it. Showing the examples of how to resolve new challenges, they pointed out and highlighted that the main challenge was to anticipate how rapidly evolving systems might go wrong or could be abused and to prevent these possible outcomes before they occur [8]. While establishing correct rules of practice for AI is a key to its proper implementation in hospitals, correct reporting in scientific reports should not be forgotten either. At the end of 2020, a consensus statement was published on reporting trials involving AI procedures [9].

This study is a retrospective review of original articles published in the last three years in the field of radiology assessing the methods of reporting information related to the usage and ethical issues of AI procedures.

Material and Methods

Literature search

An initial list of 4,301 items was generated by PubMed engine through the review of literature published over the last three years (from 2018 to 2020). When analysing the number of publications during the previous ten years (from 2010 to 2020), we noticed that more than 65% of articles were published in the last three years; hence we decided to limit our analysis to this period. The search was performed on 20 October 2020 using the terms 'artificial intelligence', 'machine learning' and 'deep learning' to identify published original articles for AI interventions in radiology. The search excluded review studies and statement or editorial articles.

The query box used during the search was: *((Radiology) AND ((Artificial intelligence) OR (Machine Learning) OR (Deep Learning))) NOT ((Review) OR (Statement) OR (Editorial))*.

In the next step, from the initial cohort, the PubMed engine built-in filter was used to extract 51 clinical trials (CT) written in English. After reading the abstracts, we narrowed the list of publications to 30 CTs that focused directly on diagnostic or interventional radiology (we excluded articles where radiology was just a tool) [10-39]. These articles constituted the first arm of the study. The second arm included 30 non-trial (original) articles (OA) randomly sampled from the initial cohort of articles [40-69]. Figure 1 shows the flow diagram that include each step of including/excluding process of the papers to this review [70].

Scope and method of analysis

The guideline published by Liu et al. [9] was used to develop the check list that was used to

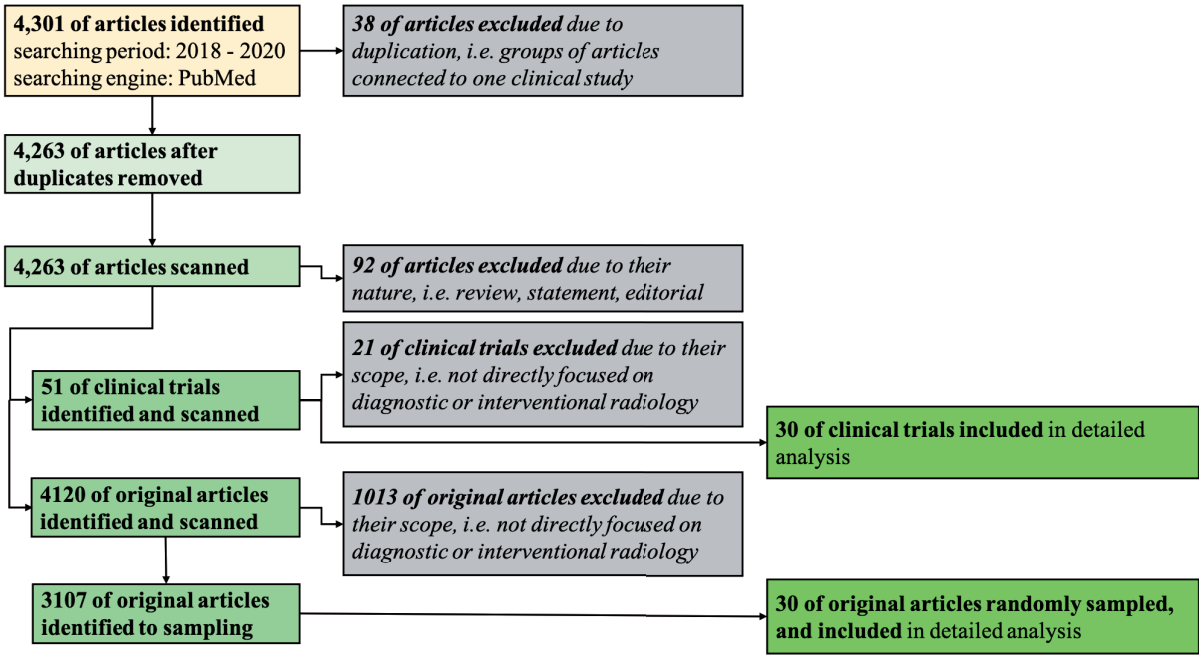

Figure 1. Flow of information through the different phases of review

Table 1. The set of items used to check the status of AI reporting in the analysed groups of articles

Item	The scope of the assessment
Q1	The title includes information on AI or, in the abstract, the use of AI intervention within the study was clearly stated.
Q2	The AI intervention was adequately justified in the context of the clinical pathway.
Q3	The inclusion and exclusion criteria at the level of input data as well as participants were stated.
Q4	Clear description of how the AI intervention was integrated into the study setting, including any onsite or offsite requirements.
Q5	Was the version of the AI algorithm stated?
Q6	Were patients informed and did they sign the consent?
Q7	Was data anonymization used and was the method described?
Q8	Were the data shared?
Q9	Description of how low quality or unavailable input data were assessed and handled.
Q10	Checking whether there was human-AI interaction in the handling of the input data, and what level of expertise was required of users.
Q11	Checking the explanations of how AI intervention outcomes contributed to decision-making or other elements of clinical practice.
Q12	How were potential harms described, i.e., description of any analysis of performance errors and how errors were identified, where applicable.
Q13	Checking whether information was provided on how AI intervention and/or its code can be accessed, including any restrictions to access or re-use.

assess accuracy, transparency and ethical issues of the AI reporting in specific parts of the article (Table 1).

Both groups (CT and OA) were independently scored for the items included in Table 1 by three observers: physicist (OBS_1), physician (OBS_2), and computer scientist (OBS_3). The assessment was made for each item on a two-stage scale (meet/fail). The Cohen's Kappa coefficient was used to measure inter-observer reliability. Separately for CT and OA, the maximum difference between observers' scores (MDO) was counted. The average percentage (AP) of the positive scores was calculated for CT and OA, and every item of Table 1. The results obtained for CT and OA were compared using Fisher's exact test. Moreover, the relative difference between AP for CT and OA was calculated.

All tests were performed at the significance level α = 0.05, using XLSTAT software (Addinsoft SARL, New York, USA) in an MS Excel environment (Microsoft Corp., Redmond, WA, USA).

Results

The analysis includes articles prepared 'on the eve' of the publication of Liu *et al*. guidelines [9]. Therefore, the criteria used to assess the AI reporting in the studied articles were not available for the authors of the cited studies at the time of publications.

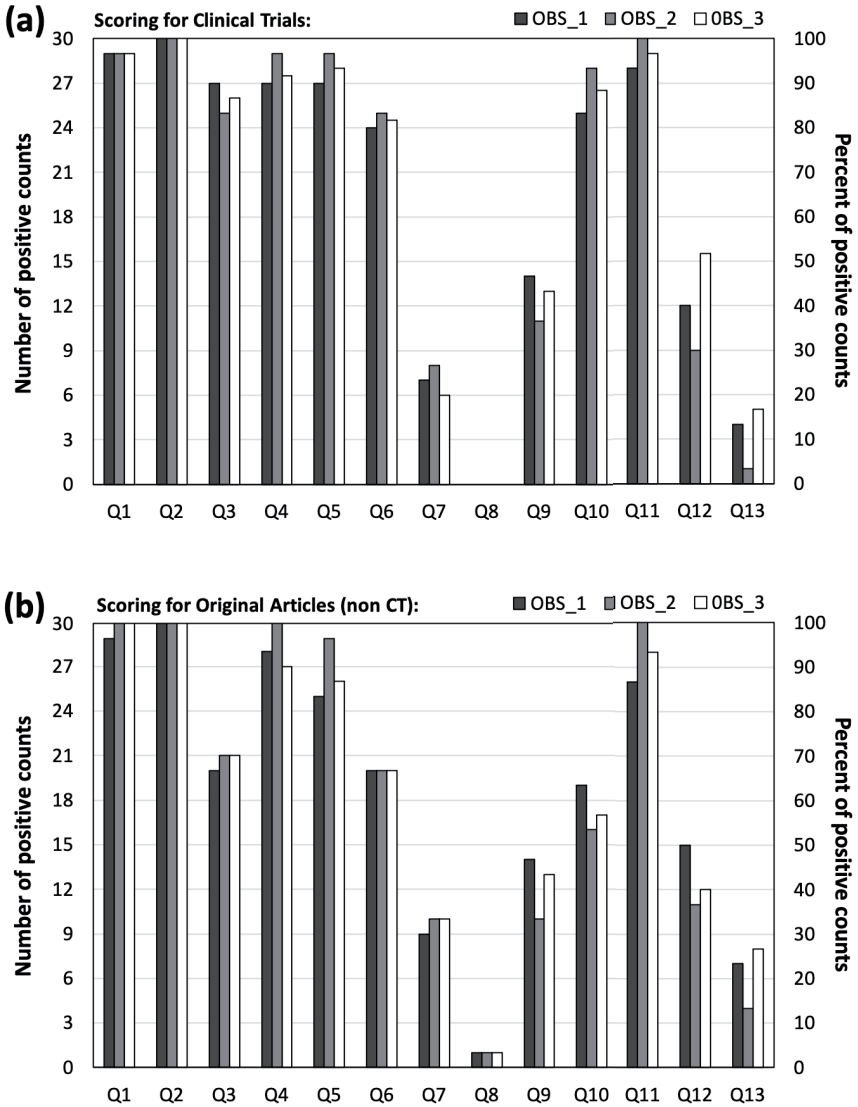

Figure 2. Positive counts from three observers related to (a) clinical trial and (b) original articles. The value of Q8 for clinical trials (a) was zero for each observer. Abbreviations: Q1-Q13 – the items described in Table 1; OBS_1 – first observer (physicist); OBS_2 – second observer (physician); OBS_3 – third observer (computer scientist)

Figure 2 shows the scores granted by every observer for CT (Figure 2a) and OA (Figure 2b) groups. The highest MDOs was 4 (13%) for the CT as well as for the OA group. While, in the CT group, these MDOs were connected to Q12 and Q13 items, in the OA group they were also linked to Q5, Q9, and Q11. The analysis of Cohen's Kappa coefficients (Figure 3) confirmed the lowest agreement between observers' scoring for Q5, Q11, Q13. Small Cohen's Kappa value was also observed for Q4 where MDO's were relatively high (i.e., 7% for CT and 10% for OA). All obtained

Item	OBS_1 *vs* OBS_2	OBS_1 *vs* OBS_3	OBS_2 *vs* OBS_3
Q1	0.66	0.66	1.00
Q2	1.00	1.00	1.00
Q3	0.90	1.00	0.95
Q4	0.31	1.00	0.31
Q5	0.37	0.84	0.47
Q6	0.96	1.00	0.96
Q7	0.92	1.00	0.92
Q8	1.00	1.00	1.00
Q9	0.76	0.93	0.83
Q10	1.00	0.96	0.96
Q11	0.27	0.64	0.49
Q12	0.76	0.97	0.73
Q13	0.58	0.90	0.50

The scale of agreement quality according to Kappa values:

0.8 - 1.0	almost perfect
0.6 - 0.8	substantial
0.4 - 0.6	moderate
0.2 - 0.4	fair agreement
0.0 - 0.2	small

Figure 3. Cohen's Kappa coefficients for agreement of the judges' answers. Abbreviations: Q1-Q13 – the items described in Table 1; OBS_1 – first observer (physicist); OBS_2 – second observer (physician); OBS_3 – third observer (computer scientist)

Table 2. The maximum differences between observers' scores and the relative difference between the average percentage of the positive scores for clinical trial and original articles groups. Statistical comparison performed by Fisher's exact test on the significance level equal to 0.05

Item	MDO		$\lvert AP_{CT} - AP_{OA} \rvert$	Fisher's exact test
	CT	OA		
	Values in numbers and (%)			
Q1	0 (0%)	1 (3%)	2%	p = 0.621
Q2	0 (0%)	0 (0%)	0%	p = 1.000
Q3	2 (7%)	1 (3%)	18%	p = 0.007
Q4	2 (7%)	3 (10%)	2%	p = 0.767
Q5	2 (7%)	4 (13%)	4%	p = 0.433
Q6	1 (3%)	0 (0%)	15%	p = 0.028
Q7	2 (7%)	1 (3%)	9%	p = 0.244
Q8	0 (0%)	0 (0%)	3%	p = 0.246
Q9	3 (10%)	4 (13%)	1%	p = 1.000
Q10	3 (10%)	3 (10%)	31%	p < 0.001
Q11	2 (7%)	4 (13%)	3%	p = 0.497
Q12	4 (13%)	4 (13%)	2%	p = 0.881
Q13	4 (13%)	4 (13%)	10%	p = 0.104

MDO - the maximum difference between observers' scores; CT - the group including Clinical Trials; OA - the group including Original Articles that are not CT; AP - the average percentage of the positive scores

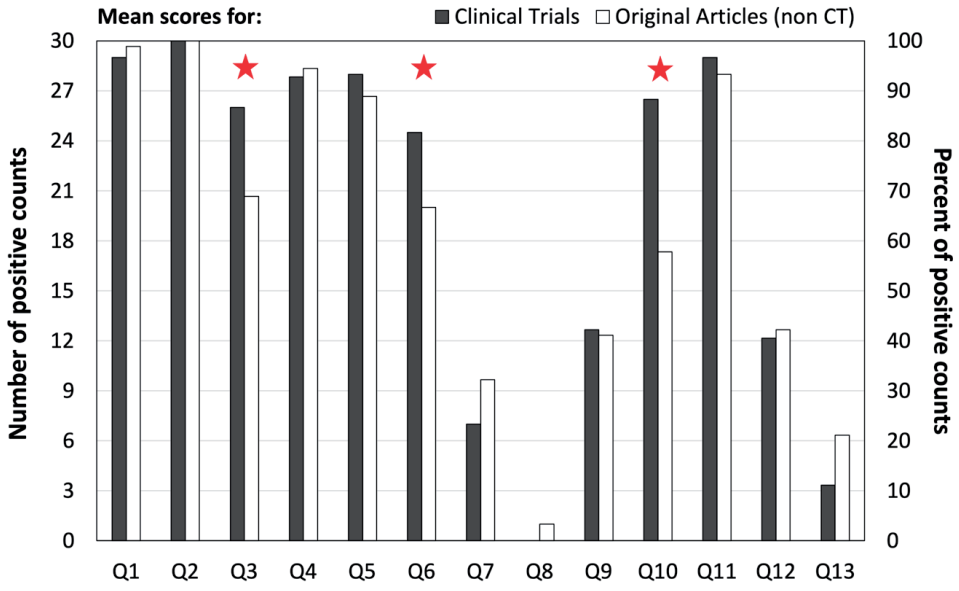

Figure 4. Averaged positive counts for the groups of clinical trial and original articles. Abbreviations: Q1-Q13 – the items described in Table 1. Red asterisk: statistically significant difference between scores granted to clinical trials and original articles

Cohen's Kappa values ranged on the scale proposed by Landis and Koch [71] from "*fair agreement*" to "*almost perfect*" level.

Table 2 shows detailed information of the MDOs in the CT and the OA groups, the relative differences between the average percentage of the positive scores counted in CT and OA, and the statistic results of CT *vs* OA comparison for every item from Table 1.

Figure 4 shows the AP of positive counts for the CT and the OA. The results lower than 50% of the passing checks in both groups were noted for five items - Q7, Q8, Q9, Q12, and Q13. While for Q3, Q6, and Q10 the passing checks were above 50% (ranged from 58% to 88%), the AP counted for CT and OA differed significantly among themselves (Fisher's exact test performed at α=0.05) (Table 2). The highest passing checks (> 90%) with smallest differences between CT and OA were observed for Q1, Q2 and Q11.

By analysing the items, we noted that only for Q7 and Q8, the passing checks were higher for OA than CT. It should be noted that the Q8 item was scored as incorrect with only one article assessed as meeting those criteria.

Discussion

The checklist proposed by Liu *et al*. [9] puts forward important criteria for safe and effective integration of AI into clinical practice, defining clear criteria of study design, data management and patients' rights to privacy. The criteria list used to score the articles, presented in Table 1, was based directly on the checklist of Liu et al. and contains all the key criteria presented by them. Although not validated yet, such proposal is a good starting point for future guidelines for authors and editors, regarding minimal standard criteria for publication of studies integrating AI tools. The assessment criteria used were not published at the time of publication of reviewed articles. Therefore, the results of our study should be interpreted as an indication of areas where the authors of future AI articles should put higher attention, based on published Liu recommendation.

Most of the reviewed studies, as expected, clearly stated their use of AI methods and, more importantly, almost all tried to address relevant clinical questions (Q1, Q2). Although in most of the studies patient inclusion and exclusion criteria were presented, they lacked widespread rigorous descriptions of the study design (Q3, Q4, Q10, Q11) apart from a detailed explanation of the AI approach itself (Q5). These concerns fit the broader discussion about transparency and reproducibility in AI research which includes reporting of data selection and flow. Additionally, using data collected in routine clinical practice - as opposed to highly curated datasets, e.g. from clinical trials - can produce the 'garbage-in, garbage out' phenomenon due to low quality or missing data points, creating the risk of wrong clinical decision. Unfortunately, this problem is rarely addressed in the reviewed publications (Q9).

A small discrepancy in the evaluation between a clinical observer (OBS_2) and a technical-scientific observer (OBS_1, OBS_3) for the items (Q9, Q10, Q11, Q12, Q13), do not change the final assessment of the quality of the articles analyzed. Rather, a possible hypothesis for future research studies is that different level of perception of ethics among different observers could have an impact on patient data and study management when applied to clinical use.

Another important part of every study protocol is informed consent of the participants (Q6). Many studies reported that local Institutional Review Board (IRB) had waived patients' consent. It can be understood when the study uses retrospective data but for prospective trials, even when images are the only subject of research, such consent should be mandatory. Very few of the chosen studies provided information about anonymization of data and process for safe sharing (Q7, Q8). The discussion regarding data ownership is still ongoing, and it is not clear who should be responsible for the evaluation of trade-off between the potential benefit for future patients and privacy concerns when patient data is released. Is patients' consent needed for sharing their data publicly or with non-medical companies or is IRB judgement sufficient? With the increasing involvement of non-medical technology companies like Google or Facebook in healthcare and the associated quest for sensitive medical data, these questions will become even more relevant in the near future [72,73]

Only a few studies explore the patterns of incorrect predictions by the proposed AI tools and possible reasons. Such analysis is impor-

tant for the evaluation of model performance as every error carries a potential cost and risk for the patient and the clinician who is fully responsible for the decision made or augmented by an AI model (Q12).

To gain the trust of clinicians, AI tools designed for use in clinical routine should be robust and transparent. Studies proposing these tools must be transparent and reproducible. According to 2020 State of AI report [74], only 15% of AI studies made the code used to train and validate the proposed models publicly available. This is clearly seen in the set of studies included in this review as only five (5%) of them are accompanied by an open-source code and metadata (Q13). The international research community have recently raised a concern about the replicability of AI research regarding a publication on an AI tool for breast cancer diagnosis built by Google researchers with no open access to the code [72]. In their comments, the authors argue that sharing key materials, like code and metadata, would allow verification of results by other scientists. Without this, published results are rather like a "promotion of closed technology" [75].

Conclusion

Recommendation on how to report results of studies with use or development of AI tools are important and should be implemented by authors and editors to increase robustness and replicability of their work. The review shows that authors of studies using AI tools should put more emphasis on the accurate description of the study design to increase transparency and reproducible of their works.

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

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THOUSAND WORDS ABOUT...

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Effectiveness of different dietary strategies in the management of obesity and obesity-related comorbidities

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ABSTRACT

The prevalence of obesity has been increasing worldwide; however, the optimal dietary strategy for improving anthropometric and cardiometabolic parameters remains unknown. This review discusses the effectiveness of popular diets in the management of obesity and obesity-related comorbidities. The differences among popular diets are small and associated with dietary adherence and caloric intake. The Mediterranean diet is most effective in facilitating weight loss and improving cardiometabolic parameters, although the Central European diet seems to be a good alternative.

Introduction

Obesity is one of the most vital public health issues, with the global prevalence nearly tripling between 1975 and 2016 and more than 650 million adults currently obese [1]. Diet plays an essential role in the prevention and treatment of obesity, although the optimal dietary strategy for improving anthropometric and cardiometabolic parameters remains unknown [2]. This review discusses the effectiveness of popular diets in the management of obesity and obesity-related comorbidities.

Mediterranean diet

The Mediterranean diet is based on the traditional cuisine of countries bordering the Mediterranean Sea and is characterised by a high intake of vegetables, fruits, grains, legumes and nuts. The main components of this diet also include a regular intake of fish, moderate intake of dairy products, limited intake of red meat and red wine, in addition to the use of olive oil as the main source of fat [3]. The efficacy of the Mediterranean diet has been investigated in a number randomised controlled trials [4–6]

and meta-analyses [2,7], revealing that this diet supports weight loss and improves cardiometabolic markers (e.g. glucose and insulin levels, inflammatory markers) [6,8]. In an umbrella review by Dinu et al. [2], the Mediterranean diet has been reported to provide the strongest and most consistent evidence of exerting a beneficial effect on anthropometric parameters (body weight and body mass index (BMI)), lipid profile, glucose and insulin homeostasis, as well as on blood pressure. Importantly, these effects seem to be independent of the caloric intake and are proportional to the dietary adherence rate [4,5].

Central European diet

The Central European diet is based on foods including grains (e.g. rye, oat), fish (e.g. herring), vegetables (e.g. beetroot, cabbages) and fruits (e.g. berries, apples, plums) [5]. Consequently, the diet is low in fat and high in dietary fibre and has been shown to significantly improve body weight, waist circumference, visceral fat, metabolic and atherosclerosis parameters. Moreover, the effectiveness of the Central European diet seems to be similar to the Mediterranean diet [5,9].

DASH diet

The dietary approaches to stop hypertension (DASH) diet is characterised by a high intake of vegetables, fruits, whole grains and low-fat dairy products, with a moderate intake of fish, poultry and nuts and a high intake of dietary fibre, calcium, magnesium and potassium. Furthermore, it includes a low intake of sodium and fat [10]. The DASH diet has been designed to control hypertension [11]. However, the diet also helps to reduce body weight [12] and decreases the risk of cardiovascular diseases [13]. In their umbrella review, Dinu et al. [2] found suggestive evidence that the DASH diet could improve weight and blood pressure. Additionally, according to a meta-analysis by Soltani et al. [12], the DASH diet is more effective in body weight reduction than other low-calorie diets, therefore, it is thought to be a good choice with regard to obesity management.

Vegetarian diet

A plant-based diet focuses on foods of plant origin comprising a high intake of fruits, vegetables, grains, legumes, nuts and oils. Depending on the type of diet, dairy products, eggs and fish may be included or excluded [14]. Recently, it has been suggested that a vegetarian diet is more effective in body weight reduction than a non-vegetarian diet, although the effectiveness may vary depending on the type of diet. In their meta-analysis, Huang et al. [15] demonstrated a significant weight reduction in subjects consuming a vegan diet and a lacto-ovo-vegetarian diet. However, a recent umbrella review has reported this diet to have a low effectiveness in reducing cholesterol and glucose levels, blood pressure and anthropometric parameters [2], possibly due to the high variability among vegetarian diets.

Low glycaemic index diet

Reducing the glycaemic index may play a crucial role in the prevention and treatment of obesity. A low-glycaemic-index diet can regulate anthropometric parameters (e.g. BMI), promote satiety and reduce food intake, as well as reduce postprandial insulin secretion, affect insulin sensitivity and maintain glucose levels within the normal range [16]. Compared to a high-glycaemic-index diet, not only does a low-glycaemic-index diet reduce body weight, but it also affects body composition [17]. Nevertheless, the umbrella review only provided suggestive evidence of a reduction in anthropometric parameters following this diet and contrasting evidence with regard to its effect on lipid and glucose levels, as well as on blood pressure [2].

Ketogenic diet

The ketogenic diet is a type of low-carbohydrate diet, in which carbohydrate intake is limited to 5-10% of the total daily dietary requirements [18]. The diet is employed in epilepsy, Alzheimer's disease [18], or autism [19]. A recent meta-analysis has also reported a ketogenic diet to be more effective in improving metabolic and anthropometric parameters in obese subjects than a low-

fat diet [20]. However, in another meta-analysis, Lee et al. [21] found no effect of combined exercises and ketogenic diet on body composition, fasting glucose and cholesterol levels. Additionally, the long-term use of the diet may be associated with an increased risk of various chronic diseases. In fact, Mazidi et al. [22] observed that participants with the lowest carbohydrate intake have the highest risk of overall mortality, as well as cardiovascular disease and cancer mortality. Moreover, several adverse effects were reported, including constipation, headache, diarrhoea, malaise and rash [23].

High-protein diet

A high-protein diet is defined as a diet in which at least 20% of energy is obtained from protein [24]. Mitra et al. [25] reported that in comparison with a standard diet, a high-protein diet significantly reduces anthropometric parameters (body weight, BMI, waist circumference, fat mass and percentage of body fat), insulin resistance, and C-reactive protein levels. Furthermore, de Luis et al. have reported a beneficial effect of a high-protein diet on anthropometric and metabolic parameters [26]. Such a positive effect of the diet on the abovementioned parameters is a consequence of the diet-induced diuresis, and is associated with glycogen mobilisation and loss of appetite. However, it should be noted that a long-term use of this diet may cause renal, bone and hepatic abnormalities [24].

Conclusions

Studies on the effectiveness of different dietary strategies in the management of obesity and obesity-related complications have shown heterogeneous findings. The differences among popular diets are small and associated with dietary adherence and caloric intake. Nevertheless, most evidence has supported the effectiveness of the Mediterranean diet in facilitating weight loss and improving cardiometabolic parameters. In view of the recent data, the Central European diet could be a good alternative to the Mediterranean diet, at least in Central European countries.

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Contributors
M.J. wrote the manuscript. M.W.G. discussed the manuscript. J.W. commented on the manuscript. All authors reviewed and approved the final manuscript.

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THOUSAND WORDS ABOUT...

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Palaeolithic diet in the treatment of type 2 diabetes

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ABSTRACT

The prevalence of type 2 diabetes has steadily increased over the past few decades. In the treatment of this disease, lifestyle modifications and dietary management are essential. There is evidence suggesting a beneficial impact of the Palaeolithic diet on monitoring glucose and insulin homeostasis; however, other studies have not confirmed these results. Therefore, further well-designed trials are necessary to demonstrate the health benefits of Palaeolithic nutrition in subjects with type 2 diabetes.

Introduction

Type 2 diabetes mellitus is a complex metabolic and endocrine disorder resulting from the interaction between genetic and environmental factors, which cause different degrees of alteration in insulin functionality on peripheral tissues, as well as in the pancreatic β cell. Over time, diabetes may lead to long-term complications, such as neuropathy, nephropathy and retinopathy. Type 2 diabetes is strongly associated with obesity, and subjects with diabetes are at a higher risk of developing cardiovascular disease, or non-alcoholic fatty liver disease. These complications can be delayed, or prevented by appropriate management of diabetes [1-3]. An important part of the treatment of this disease is diet. The American Diabetes Association (ADA) suggests there is no ideal eating pattern, or macronutrient distribution for individuals suffering from diabetes. However, subjects with diabetes should choose non-starchy vegetables, reduce added sugars and refined grains and replace highly processed foods with natural foods [4].

The Palaeolithic diet, also known as the Paleo diet, the stone-age diet, or the hunter-gatherer diet, is based on the dietary patterns of our ancestors who lived during the Palaeolithic era. This diet consists of eating vegetables, fruits, meat, fish, eggs and nuts, while excluding dairy products, refined grains, oils and legumes [5]. It is often classified as a low-carbohydrate diet which provides a low amount of sodium, and a high amount of dietary fibre, potassium and antioxidants [6]. Naturally, using the Paleo diet nowadays has some limitations, since it is not possible to fully adopt the same diet as people did 10,000 years ago. Nevertheless, it can be concluded that both the Palaeolithic diet and the nutritional therapy recommended by the ADA are based on similar products. Recently, the Paleo diet has become popular due to its health benefits, such as reduc-

ing anthropometric parameters, or improving lipid profiles and blood pressure [6-8]; however, the effectiveness of this diet in the treatment of type 2 diabetes remains unclear.

The effect of the Palaeolithic diet on glucose and insulin levels

Studies have shown that a Palaeolithic-type diet may improve fasting glucose and insulin concentrations [9, 10]. Lindeberg et al. [11] conducted a 12-week study in adults with ischemic heart disease and either glucose intolerance, or type 2 diabetes. They observed that 2-hour plasma glucose concentrations following an oral glucose tolerance test (OGTT) decreased by 26% in the Paleo diet group, whereas in the Mediterranean diet group the 2-hour plasma glucose levels decreased only by 7%. Similarly, Jönsson et al. [12] observed a significant reduction in the area under the curve (AUC) between 0 and 120 minutes (AUC 0–120) for glucose levels after subjects followed a Palaeolithic diet for 3 months. Moreover, Frassetto et al. [13] demonstrated a reduction in AUC 0–120 for insulin levels in the Palaeolithic diet group after only 10 days of the intervention. Unfortunately, Otten et al. [14] have observed no differences between the effects of the stone-age diet and the Nordic Nutrition Recommendations (NNR) on AUC 0–120 for glucose and insulin in the course of the OGTT. In two meta-analyses, the effects of the Palaeolithic diet on glucose and insulin concentrations were also not found [15, 16].

The effect of the Palaeolithic diet on insulin resistance and sensitivity

Elevated fasting glucose and insulin levels are associated with insulin resistance, as measured by the homeostasis model assessment of insulin resistance (HOMA-IR) index [17]. During a 2-year intervention, Otten et al. [18] showed a significant improvement in the HOMA-IR index after a 6-month Palaeolithic diet intervention, although this improvement deteriorated significantly between 6 and 24 months. Another study conducted by Otten et al. [9] also demonstrated that insulin resistance improved by 45% in subjects with type 2 diabetes on the Palaeolithic diet. In addition, a positive effect of the Palaeolithic diet on insulin resistance was also obtained in several other studies [6, 8, 19]. Nevertheless, a recent meta-analysis failed to confirm a significant effect of Paleo nutrition on the HOMA-IR index, and it was described as having no effect on fasting glucose and insulin concentrations [15]. On the other hand, Masharani et al. [6] reported that the Paleo diet improves insulin sensitivity in most insulin-resistant patients, which was not observed in the case of a diet adhering to ADA recommendations. Moreover, even following the Paleo diet over a short period (14 days) positively affected glucose control and lipid profiles [6].

The effect of the Palaeolithic diet on HbA1c levels

Glycated haemoglobin (HbA1c) levels provide information concerning the average blood glucose levels for the past 2 to 3 months and are a commonly used indicator of the metabolic control of diabetes [4]. A 3-month randomised crossover study demonstrated that in the Paleo group HbA1c levels were significantly lower (-0.4%) than in individuals following the conventional diabetes diet [12]. However, in a 12-month trial, participants following the Mediterranean diet demonstrated a greater reduction in HbA1c than those in the Palaeolithic diet group [20]. Nevertheless, the varying duration periods of the interventions may partially account for the differences in the obtained results.

The effect of the Palaeolithic diet on anthropometric parameters

It is suggested that the effectiveness of a diet in reducing glucose and insulin levels may depend on its effect on body weight reductions and other anthropometric parameters [21]. Meta-analyses conducted by Menezes et al. [7] and Ghaedi et al. [22] confirmed the effect of the hunter-gatherer diet on decreasing anthropometric parameters, such as body weight, waist circumference and body mass index. In a 2-year randomised controlled trial including obese women, greater ben-

eficial effects on anthropometric parameters were reported following the consumption of the Paleo diet in comparison with a diet based on the NNR [23]. Furthermore, in a similar long-term intervention, Stomby et al. [19] found that weight loss in overweight or obese women following the Palaeolithic diet was greater than those on the NNR diet after 6 months, although not at 24 months. Fasting serum insulin levels also decreased at 6 months in both groups, but the insulin concentrations were more favourable in the Paleo diet group.

Conclusions

In conclusion, the Palaeolithic diet may have powerful beneficial metabolic and physiologic effects in type 2 diabetes. However, the results of the available studies evaluating the effects of Palaeolithic nutrition on glucose and insulin homeostasis are not conclusive. Therefore, well-designed long-term trials are still necessary to confirm the effectiveness of the Palaeolithic diet in subjects with type 2 diabetes.

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

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