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ORIGINAL PAPER

JMS Journal of Medical Science

A study to assess the effect of pretreatment with intravenous palonosetron in preventing pain on propofol injection

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ABSTRACT

Background. Propofol is widely used for induction and maintenance of anaesthesia and possesses many characteristics of an ideal intravenous anaesthetic agent. It is known to cause severe, sharp, stinging, or burning pain on injection, which is considered unacceptable as it can cause agitation and interfere with the smooth induction of anaesthesia. In this study, we compare palonosetron and normal saline for decreasing pain on injection of propofol during intravenous induction of anaesthesia.

Material and methods. One hundred adult patients belonging to ASA physical status I or II, scheduled for elective surgeries under general anaesthesia, were selected and randomly allocated to two groups. Group P received an injection of palonosetron, and Group S received an injection of regular saline as a pretreatment before the propofol injection. Patients were assessed for pain on propofol injection. Haemodynamic parameters and electrocardiography were recorded at the following points of time: prior to induction, after pretreatment, induction, and half-hourly during the surgery.

Results. Comparing pain during propofol injection, 32% of the palonosetron group and 4% of the regular saline group did not experience pain; 54% of the palonosetron group and 20% of the regular saline group suffered mild pain; 12% of the palonosetron group and 48% of the regular saline group developed moderate pain; 2% of the palonosetron group and 28% of the regular saline group experienced severe pain.

Conclusions. Pretreatment with palonosetron 0.075 mg reduced the incidence and severity of propofol-induced pain on injection, with the added advantage of decreased postoperative nausea and vomiting without significant haemodynamic changes.

Introduction

Propofol is the most commonly used intravenous induction agent today. The formulation commonly used is that of 1% propofol, 10% soyabean oil, and 1.2% purified egg phospholipid added as emulsifier, with 2.25% glycerol as a tonicity- adjusting

agent and sodium hydroxide to change pH [1]. It is the drug of choice for induction of anaesthesia in a lot of patients due to its rapid onset, short duration of action, easy titration, and favourable side effects profile [2]. Induction with propofol is associated with pain on injection, apnoea, hypotension, and, rarely, thrombophlebitis of the vein into which propofol is injected [1]. In various studies, the incidence of pain is about 60% on injection of propofol without any preventive measures [3]. The mechanism for pain on injection of propofol is unclear. However, it has been postulated that it could be associated with a direct or indirect irritant effect by releasing pro-inflammatory mediators [4]. The initial component of pain involves immediate stimulation of nociceptors and free nerve endings, mainly associated with the concentration of free drugs within the aqueous phase of the emulsion [5].

Several studies have demonstrated that 5-hydroxytryptamine (5-HT3) receptor antagonists could reduce the incidence of propofol injection pain [4, 6]. Peripheral 5-HT3 receptors are known to be involved in the nociceptive pathway. 5HT3 receptor antagonists could be used as a local anaesthetic based on their effect in blocking sodium channels. Palonosetron is a second-generation 5HT3 receptor antagonist, which reduces pain on propofol injection and decreases postoperative nausea and vomiting. Hence, the study performed to assess and evaluate the effectiveness of palonosetron in reducing the occurrence of propofol–induced pain.

The study's primary objective was to assess the effect of pretreatment with intravenous palonosetron in preventing pain on propofol injection. A secondary objective was to evaluate the safety profile associated with using palonosetron and assess the duration of action in reducing postoperative nausea and vomiting..

Material and methods

After obtaining institutional ethical committee clearance and written informed consent from patients, a prospective, randomised, controlled single-blind study was conducted on 100 patients aged 18–60 yrs, belonging to ASA (American Society of Anesthesiologists) grade I & II, who were scheduled for surgeries under general anaesthesia. The trial was registered in UMIN UMIN000050665.

Patients who cannot verbally express the severity of pain, ischemic heart disease, previous myocardial infarction, congestive heart failure, congenital long QT syndrome, electrolyte abnormalities, hepatic and renal dysfunction, chronic alcohol abuse and patients on antipsychotic drugs were excluded from the study groups. Patients were randomly allocated to one of the two groups using numbers from www.random.org. Group P – Injection Palonosetron 0.075 mg (2 ml) whereas Group S – Normal Saline (2 ml). Allocation concealment was ensured using sequentially numbered sealed envelopes, opened after moving the patient to the operation table.

All patients were assessed preoperatively, given study details, and informed about the anaesthetic procedure they were to undergo. Patients were kept fasting for 8 hours prior to their scheduled surgery. Alprazolam 0.5 mg and Ranitidine 150 mg were given orally the previous night of surgery. On the day of surgery, intravenous (IV) access was established, and an IV infusion of Ringer lactate was started.

All the patients were premedicated with an injection of Glycopyrrolate 0.005 mg/kg IV, an injection of Midazolam 0.03 mg/kg, followed by respective study drugs and anaesthesia was induced with propofol as mentioned below.

Patients were informed regarding pain on propofol injection. Patients in each group received respective drugs, followed by anaesthesia induction with propofol after 3 minutes, as already mentioned.

Patients were preoxygenated, and the venous drainage of the limb was occluded after giving the study drug by applying a tourniquet inflated to 70 mmHg for 1 min, after which 25% of the total calculated dose of propofol injection (2 mg/kg) was given over 5 seconds and assessed for degree of pain [7].

Monitoring included electrocardiography (ECG), peripheral oxygen saturation (SpO2), non-invasive blood pressure (NIBP), end-tidal carbon dioxide (EtCO2), and train of four (TOF). Monitors were connected to patients, baseline haemodynamic parameters were recorded, and Qt interval was noted during premedication, pretreatment of the study drug and half hourly till the end of surgery. Patients were induced

with remaining propofol followed by vecuronium 0.1 mg/kg IV. After 3 minutes, intubation was done with an appropriate-sized endotracheal tube. An injection of fentanyl 2 µ/kg was given after intubation. Anaesthesia was maintained with oxygen at 33%, nitrous oxide at 66%, and isoflurane at 1–2%, which was titrated to maintain haemodynamic parameters within 20% of basal readings. Adequate muscle relaxation was ensured by maintaining TOF count < 2 with intermittent injections of vecuronium 0.02 mg/ kg. At the end of the surgery, muscle relaxation was reversed with Glycopyrrolate 10 mcg/kg and Neostigmine 0.05 mg/kg IV, and patients were extubated when the TOF ratio was > 0.9.

In both groups, haemodynamic parameters (heart rate, systolic blood pressure, diastolic blood pressure, mean arterial pressure, SpO2) were continuously monitored and recorded every 5 min till the end of surgery. Haemodynamic changes and intraoperative blood loss guided intraoperative fluid management. Postoperatively, intravenous fluids, antibiotics and other medications were administered per standard institutional protocol.

Nausea and vomiting were monitored during the immediate postoperative period, early (0–2 hrs), and late postoperative period (2–24 hrs), and an injection of ondansetron 4 mg intravenously was administered if there was nausea and vomiting.

The patients who suffered postoperative nausea and vomiting received ondansetron postoperatively as a rescue measure.

The sample size was calculated based on a previous study [9]. Pain incidence on propofol injection without preventive measures was about 60% [9]. With α value of 0.05 and power of 80%, the sample size calculated will be 43 patients to detect at least a 50% difference between the regular saline and palonosetron groups concerning propofol-induced pain. For an α value of 0.01 and better validation, each group comprised 50 patients.

Data was entered into a Microsoft Excel data sheet and analysed using SPSS version 22 software. The present study applied descriptive and inferential statistical analysis. Results on continuous measurements were presented as mean ±SD (Min-Max), and categorical measurements were presented in number (%). Student t-test (two-tailed, independent) was used to find the significance of study parameters on a continuous scale between two groups (Intergroup analysis) on metric parameters. The Chi-square/ Fisher Exact probability test was used to find the significance of study parameters on a categorical scale between two or more groups.

Statistical software: The statistical software, namely SPSS 15.0, was used to analyse the data, and Microsoft Word and Excel were used to generate graphs and tables.

Results

Figure 1 shows that a hundred patients were considered for the analysis. There were no dropouts. Demographic characteristics and duration of surgery in both groups were comparable. In both the palonosetron and regular saline groups, the age distribution ranged from 18–60 years, with a mean age for the palonosetron group being 40.14 ± 10.09 and for the regular saline group being 41.50 ± 9.68. The difference in age between both groups is not statistically significant. The sex difference between the groups is statistically insignificant. The mean weight in the palonosetron group is 52.10, and the mean weight in the regular saline group is 50.76. The difference between the two groups in the distribution of patients' weight is insignificant (see **Table 1**).

In our study, 52% of patients in the palonosetron group and 46% of patients in the regular saline group belong to ASA I, 48% of patients in the palonosetron group and 54% of patients in the regular saline group belong to ASA II (see **Table 1d**). The difference between the two groups regarding the distribution of ASA physical status is insignificant. The mean duration of surgery in the palonosetron group was 2.33 ± 0.7 hrs, and in the regular saline group, it was 2.59 ± 0.75 hrs (see **Table 1e**).

Comparing pain during propofol injection, 32% of the palonosetron group and 4% of the regular saline group did not suffer pain; 54% of the palonosetron group and 20% of the regular saline group experienced mild pain; 12% of the palonosetron group and 48% of the regular saline group developed moderate pain; and 2% of the palonosetron group and 28% of the regular saline group experienced severe pain. Palonosetron

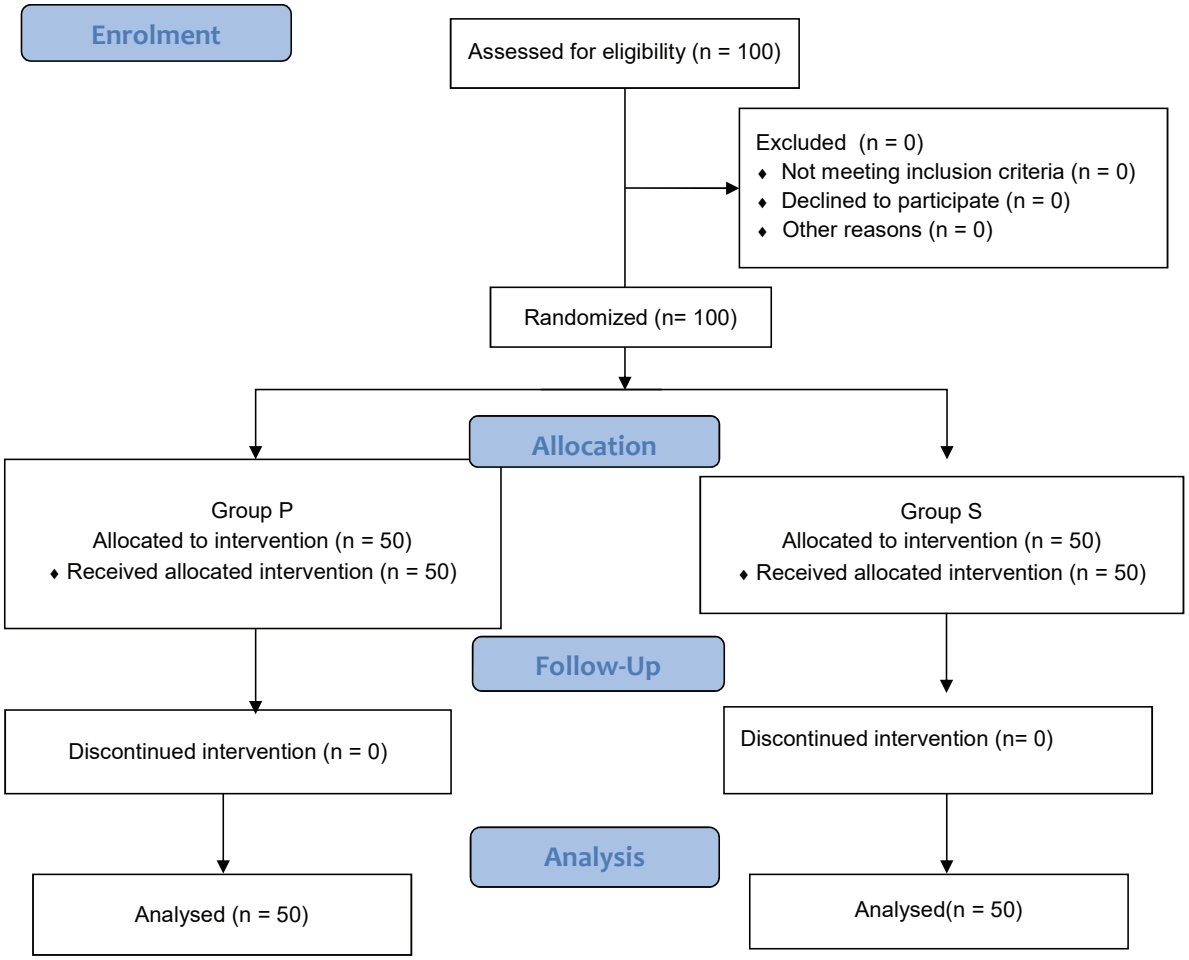

Figure 1. CONSORT Flow Diagram.

Table 1a. Demographic characteristics in the study groups included. Age distribution of cases in study groups.

Age in years	Group P	Group S
<20	2(4%)	0(0%)
20-30	7(14%)	7(14%)
31-40	16(32%)	15(30%)
41-50	17(34%)	16(32%)
51-60	8(16%)	12(24%)
Total	50(100%)	50(100%)
Mean ± SD	40.14 ± 10.09	41.50 ± 9.68

Samples are age-matched with P = 0.493, student t-test.

Table 1b. Demographic characteristics in the study groups included. Sex distribution of cases in study groups.

Gender	Group P	Group S	Total
Female	31(62%)	23(46%)	54(54%)
Male	19(38%)	27(54%)	46(46%)
Total	50(100%)	50(100%)	100(100%)

Table 1c. Demographic characteristics in the study groups included. Comparison of weight, height and bmi in study groups.

	Group P	Group S	Total	P value
Weight (kg)	52.10 ± 4.81	50.76 ± 5.13	51.43 ± 4.99	0.181
Height (cm)	152.18 ± 3.57	153.44 ± 3.82	152.81 ± 3.74	0.092+
BMI (kg/m^2)	22.52 ± 2.01	21.54 ± 2.28	22.03 ± 2.19	0.025*

Table 1d. Demographic characteristics in the study groups included. Comparison of asa physical status in study groups.

ASA Grade	Group P	Group S	Total
Grade I	26(52%)	23(46%)	49(49%)
Grade II	24(48%)	27(54%)	51(51%)
Total	50(100%)	50(100%)	100(100%)

P = 0.548

Table 1e. Demographic characteristics in the study groups included. Comparison of duration in study groups.

Duration of Surgery (hours)	Group P	Group S	Total
<2	9(18%)	4(8%)	13(13%)
2-4	41(82%)	46(92%)	87(87%)
Total	50(100%)	50(100%)	100(100%)
Mean ± SD	2.33 ± 0.70	2.59 ± 0.75	2.46 ± 0.73

P = 0.076+

significantly reduced pain on propofol injection (p < 0.001) (see **Table 2a**).

There was no significant change in pain score in Males and females in both Group P (P value 0.556) and Group S (P value 0.947) (see **Table 2b**).

The baseline heart rate was comparable between the groups. There was an increase in heart rate after study drug administration in Group P (87.44 ± 8.42) compared to Group S (84.44 ± 8.74), but clinically, it was insignificant (P value -0.083+).

The Systolic Blood Pressure was comparable between the groups at baseline, but after the study drug and after induction with propofol, the intragroup comparison showed a decrease in Systolic Blood Pressure in both Group P and Group S, which was statistically significant (p-value <0.001**), but clinically, it was insignificant which was treated with IV fluids. Intergroup comparison showed no significant change in systolic blood pressure in either group. The Diastolic Blood Pressure was comparable between the groups at baseline, after the study drug and after induction. The intragroup comparison showed hypotension in both groups after induction with propofol from 1–5 min (P value <0.001**), which was statistically significant but clinically insignificant (see **Figure 2**).

24% of patients in the palonosetron group had early postoperative nausea and vomiting compared to 78% in the regular saline group, and 26% of patients in the palonosetron group had late postoperative nausea and vomiting compared to 90% in the regular saline group. 76% of patients in the palonosetron group and 22% in the regular saline group did not have postoperative nausea and vomiting in the early postoperative period. 74% in the palonosetron

Table 2a. Assessment of pain on propofol injection.

McCrirrick and Hunter Pain Scale	Group P	Group S	P value
Negative response to questioning (No pain)	16 (32%)	2 (4%)	0.0001**
Pain reported in response to questioning only without behavioral signs (Mild pain)	27 (54%)	10 (20%)	0.0002**
Pain reported to questioning and accompanied by behavioural signs, or pain reported spontaneously without questioning (Moderate pain)	6 (12%)	24 (48%)	0.00004**
Strong facial grimacing, arm withdrawal or tears (Severe Pain)	1 (2%)	14 (28%)	0.0004**
Total	50 (100%)	50 (100%)	
Mean ± SD	1.14 ± 0.76	2.00 ± 0.81	

P < 0.001**

Table 2b. Sex distribution in pain on propofol injection.

Degree of pain	Group P		Group S	
	Males	Females	Males	Females
No pain	5	11	1	1
Mild pain	11	16	6	4
Moderate pain	2	4	12	12
Severe pain	1	0	8	6
Total	19	31	27	23

Group P p-value: 0.556; Group S p-value: 0.947

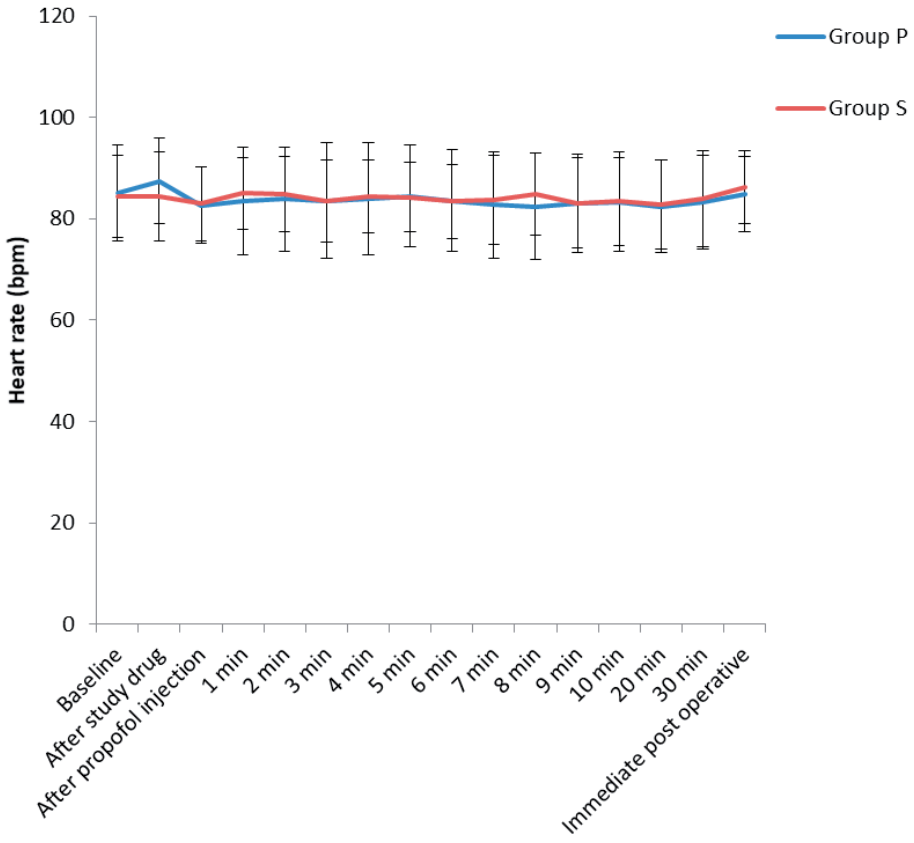

Figure 2a. Haemodynamic parameters in both groups. Line diagram showing changes in heart rate in study groups.

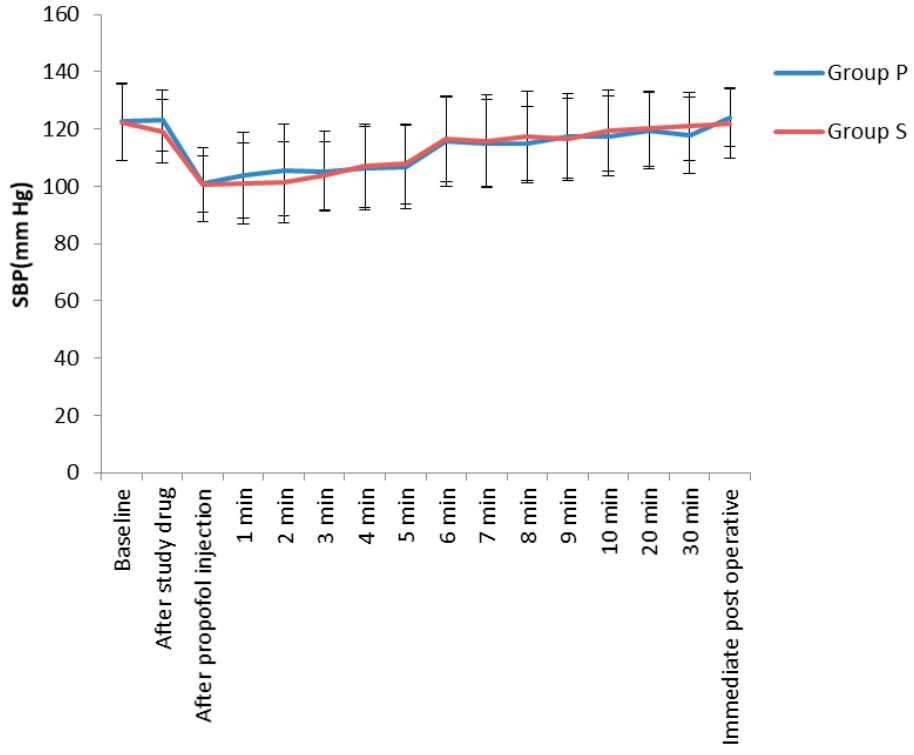

Figure 2b. Haemodynamic parameters in both groups. Line diagram showing changes in systolic blood pressure in study groups.

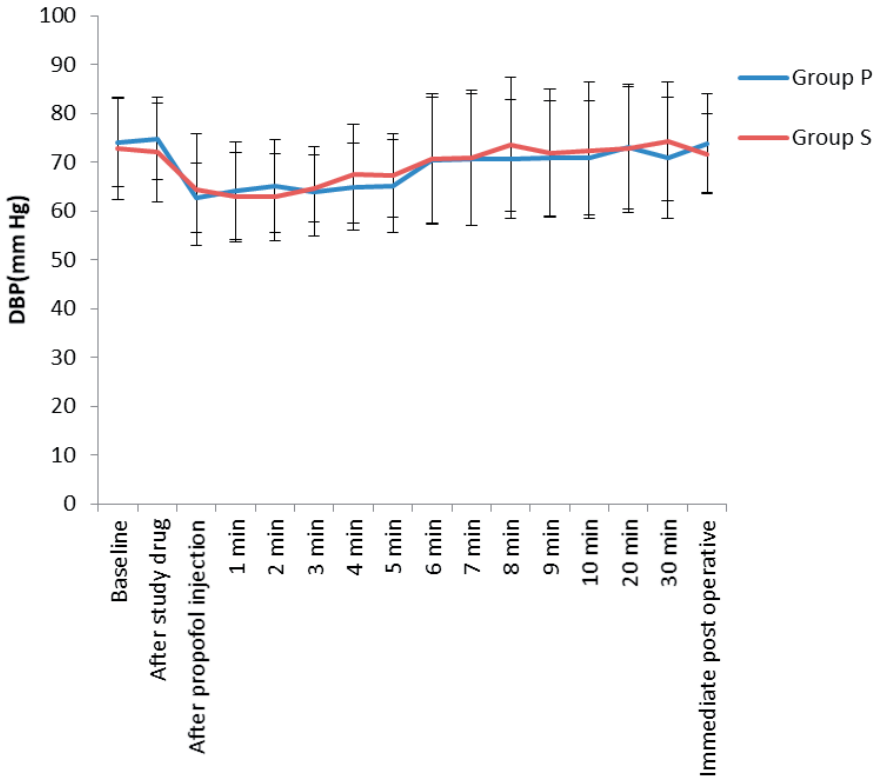

Figure 2c. Haemodynamic parameters in both groups. Line diagram showing changes in diastolic blood pressure in two groups.

Table 3. Comparison of postoperative nausea and vomiting in study groups.

PONV	Group P (n = 50)	Group S (n = 50)	Total (n = 100)	P value
Early				
– No	38(76%)	11(22%)	49(49%)	<0.001**
– Yes	12(24%)	39(78%)	51(51%)	
Late				
– No	37(74%)	5(10%)	42(42%)	<0.001**
– Yes	13(26%)	45(90%)	58(58%)	

Table 4. Comparison of qtc interval in both the groups.

Qtc. interval (msec)	Group P	Group S	P value
Premedication	376.60 ± 15.73	368.20 ± 11.37	0.003**
Pretreatment	374.60 ± 13.43	376.00 ± 10.69	0.566
Induction	368.40 ± 10.57	371.80 ± 10.04	0.102
Every 30 min	370.80 ± 9.66	371.60 ± 15.83	0.761

group and 10% in the regular saline group did not have late postoperative nausea and vomiting (see **Table 3**).

The antiemetic action of palonosetron was more than 24 hours postoperatively.

The Mean Qtc interval in the palonosetron group was 374.60, and in the regular saline group, it was 376. There was no Qt prolongation intraoperatively and postoperatively (see **Table 4**).

No incidence of Qtc prolongation, giddiness or tinnitus was noted in any patient in our study.

Though palonosetron was a known antiemetic, we assessed its duration of antiemetic actions on postoperative nausea and vomiting.

Discussion

Propofol is a fast-acting agent, and its action wears off quickly, making it useful for daycare procedures [10]. It provides excellent sedation, amnesia, anxiolysis and a state of general well-being with the added advantage of having antiemetic properties. It suppresses the upper airway reflexes in response to laryngoscopy and intubation, which is of great help in patients with hypertension, epilepsy or hyperactive airway. It attenuates stress response to intubation.

Propofol has gained tremendous popularity in daycare surgery, cardiac anaesthesia, neuro

anaesthesia and ICU sedation for its attractive profile. However, it is also associated with side effects like myoclonus, apnoea, hypotension and pain on injection [11].

The incidence of pain on injection of propofol varies from 30–90% of patients in various studies [12]. Klement and Arndt pointed out that its high osmolality and acidic pH lead to pain [13]. Other drugs, like diazepam and etomidate, also have osmolality and cause pain on injection.

Pretreatment of many drugs to decrease propofol pain on injection has been tried in different ways, i.e. either given intravenous before propofol or given with a tourniquet [14] similar to Bier's block or pretreatment drug mixed with propofol. A systematic literature search by Picard et al. found that lignocaine given with a tourniquet was the most effective method to decrease pain [8]. Other drugs which were also tried were metoclopramide [15], opioids [16] and ondansetron [17], which were found to be effective as well. Our study used a tourniquet pressure of 70 mmHg, which was maintained for one minute during pretreatment and released prior to propofol injection. Lee et al., in their study, undertook a similar method [17]. In this study, we avoided any intravenous premedication (than the study drugs), which may cause irritation or analgesia before injection of propofol.

Ryu et al. [18], Singh TH and coworkers [19], and Lee KH et al. [20] in their study found that Palonosetron 0.075 mg was effective in reducing pain on propofol injection, which was comparable to our study as we used the exact dosage and the results were similar.

Most patients in the palonosetron group experienced only mild pain and reported pain only on questioning. The finding is comparable with the study of Ambesh et al., who found that ondansetron decreased pain in almost 50% of patients. Our results also resemble the study by Kang et al., who showed that about 60% of patients did not have pain after pretreatment with ondansetron [21]. Another study conducted using microemulsion propofol found that lignocaine 2% 2 ml (52%) was more effective than ondansetron 4 mg (84%) in reducing injection pain [22]. Memis et al. compared the efficacy of tramadol and ondansetron in minimising pain due to the propofol injection in 100 patients. They showed that 4 mg ondansetron was as effective as 50 mg tramadol in preventing pain from propofol injection [23]. Singh DK, his colleagues, and Ahmed et al. used granisetron in their comparative study and found that the incidence of pain on propofol injection was scarce [6,24]. Lee and his group used ramosetron and found that it reduces pain on propofol injection [25]. Our study found that propofol injection pain was lesser in the group pre-treated with palonosetron than in the regular saline group.

Our study used propofol as an induction agent, so the incidence of PONV in both groups was scarce. 74% of the palonosetron group and 10% of the regular saline group did not develop late postoperative nausea or vomiting, which was similar to the study done by Gralla et al. [26]. Therefore, the palonosetron group had the added advantage of having less number of patients with PONV (p-value <0.001). In contrast, Lee KH et al. found no significant differences in PONV in their groups [20].

Our study compared the effect of palanosetron and placebo in decreasing propofol-induced pain. We also assessed the duration of the antiemetic potential of palonosetron, which was more than 24 hours. By this study, we infer that a single injection of Palonosetron could address both problems, such as pain on propofol injection, and reduce postoperative nausea and vomiting.

The limitation of the study was that we assessed postoperative nausea and vomiting between an antiemetic palonosetron and a placebo.

Further scope of the study is that palonosetron could be compared with ondansetron or any other antiemetic to know its potency in reducing propofol-induced pain.

Conclusions

Pretreatment with Palonosetron 0.075 mg reduced the incidence and severity of propofol-induced pain on injection, with the added advantage of decreased postoperative nausea and vomiting without significant haemodynamic changes.

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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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Lipid profile after switching from TDF (tenofovir disoproxil)-containing to TAF (tenofovir alafenamide)-containing regimen in virologically suppressed people living with HIV

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ABSTRACT

Background. Tenofovir disoproxil fumarate (TDF) or its prodrug, tenofovir alafenamide fumarate (TAF), is currently being recommended in treatment of HIV infection. The distinct pharmacological properties of these two forms of this drug make TAF treatment less nephrotoxic and lead to a better impact on bone density. Nevertheless, a rising concern about TAF's possible metabolic adverse effects exists. This study aimed to evaluate the effects on the lipid profile among ART (antiretroviral therapy) patients switching from a TDF-containing to a TAF-containing regimen in the first year after the switch.

Methods. Demographic and clinical data of HIV-positive ART-experienced patients treated in the infectious diseases department was retrospectively collected. Lipid profile change concerning baseline BMI, age, and time of ART duration were analysed.
Results. In the group of 36 patients, there was a significant increase in total cholesterol levels (+18.43 mg/dl, SD = 23.86 mg/dl, p < 0.0001) and LDL levels (+13.75 mg/dl, SD = 23.05 mg/dl, p = 0.001) in the first 12 months after switching from a TDF-containing to a TAF-containing regimen. There were no statistically significant changes in both HDL and TG levels observed. Analysis of total cholesterol and LDL levels in specific subpopulations revealed a significant increase within the first year after the switch in patients younger than 40 years old and in those whose BMI was within the normal range.
Conclusions. The data suggests that switching from TDF to TAF in ART-experienced patients may be associated with worsening lipid parameters. Early detection and management of dyslipidemias among HIV-positive patients are needed.

Introduction

The main aims of antiretroviral therapy (ART) in HIV-positive patients are undetectable viral load, reduction of transmission of the virus, restoration of the immune system and decrease in AIDS-associated mortality [1]. Effective and safe antiretroviral drug availability led to an improvement in life expectancy among people living with HIV (PLWH) to the point where it is close to the non-infected population [2,3]. Nevertheless, in the era of worldwide access to long-term treatment of HIV infection, currently, the main causes of mortality are non-AIDS-associated comorbidities such as metabolic and cardiovascular diseases [4]. The incidence of ischemic heart disease, arterial hypertension, diabetes mellitus, or dyslipidemia is significantly higher among PLWH compared to healthy individuals [5]. The pathophysiology processes leading to these observations are complex and involve endothelial dysfunction associated with the chronic inflammatory state despite suppression of virus replication, immune system dysregulation, high incidence of traditional risk factors (e.g. smoking), or side effects of drugs included in ART [6].

Tenofovir alafenamide (TAF) and tenofovir disoproxil (TDF) are two forms of tenofovir that are currently recommended in the treatment of HIV infection [7]. TAF displays non-inferior antiviral properties compared with TDF in both HIV infection treatment and pre-exposure prophylaxis [8,9]. TAF is the next-generation tenofovir prodrug with a distinct pharmacological profile. The active metabolite of these two drugs (tenofovir diphosphate) can achieve even 25 times higher concentrations in peripheral mononuclear blood cells following consumption of TAF compared with TDF [10]. Pharmacological studies have shown that tenofovir undergoes active uptake by white blood cells when its precursor is TAF [11,12]. Hence, it is possible to significantly decrease the dosage of TAF compared with TDF, leading to a better safety profile – lower nephrotoxicity risk and lesser damage to bone structural integrity [13].

Nevertheless, recent reports bring up a concern about the substitution of TDF with TAF in ART due to a possible increase in cardiovascular risk after the switch of these drugs [14]. In this single-centre retrospective study, we aimed to evaluate whether switching treatment from a TDF-containing regimen to a TAF-containing regimen is associated with worsening serum lipids parameters in the ART-experienced cohort

Material and methods

We analysed data gathered in routine care patients' charts admitted to our department. The research included patients that met the general inclusion criteria as follows: confirmed HIV infection, age over 18 years, no active neoplastic disease, switching from TDF-based regimen to TAF-based regimen, and confirmed efficacy of virologic suppression on TAF-based regimen (<200 copies/mL of HIV RNA after at least six months from treatment initiation). Patients were treated with various antiretroviral regimens that included TDF, such as TDF/emtricitabine/lopinavir/ritonavir, TDF/emtricitabine/darunavir/

ritonavir and TDF/emtricitabine/efavirenz. They were later switched to TAF-based regimens: TAF/elvitegravir/emtricitabine/cobicistat or TAF/emtricitabine/rilpivirine.

Lipid concentration measures were taken, including total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) concentration, and triglycerides (TG). These measures were taken firstly at the beginning of TAF-containing ART (at the moment of the switch from TDF-containing ART) and then 12 months after switching from TDF-containing ART. Additional information that was collected included duration of HIV infection, duration of ART, number of previous treatment schemes, route of infection, HIV RNA viral load and CD4+ count at the moment of infection diagnosis, CD4+ at the time of switching of ART regimens, co-infection with other sexually transmitted diseases (STDs). Optimal values of lipid parameters were distinguished according to Adult Treatment Panel Guidelines III and were <200 mg/dl for total cholesterol, <100 mg/dl for LDL, <150 mg/dl for TG and >40 mg for HDL in plasma serum [6].

Paired t-tests were applied to compare changes in concentration of described lipid parameters using GraphPad Prism 8.4.3 software. The statistical significance of the results was a p-value <0.05.

Results

Study population

A total of 106 patient history charts were analysed. Eventually, 36 patients met the inclusion criteria. All the patients in the study group were Caucasians and predominantly male. The median ART duration among patients was nine years, and more than half had changed their ART regimen more than two times. Importantly, we observed a high prevalence of co-infection with other sexually transmitted diseases in the study group. More than half of patients declared the most possible transmission by sexual contact and belonged to the group described as "men having sex with men". **Table 1** presents all baseline characteristics of the study group.

Lipids parameters changes in the study population

Our data indicates a high frequency of patients whose lipid parameters were not optimal at the beginning of the study at the time of the switch from TDF-containing to TAF-containing regimen. Twelve months after switching, the number of patients with different types of dyslipidemia increased from 6 to 10 for total cholesterol levels, 13 to 20 for LDL levels, 12 to 17 for TG levels, and 8 to 11 for HDL levels (**Figure 1**). Mean values of lipid parameters at the time of switching regimens were 162.5 mg/dl (SD = 36.04 mg/dl) for total cholesterol, 91.48 mg/dl (SD = 29.14 mg/dl) for LDL, 48.17 mg/dl (SD = 15.13 mg/dl) for HDL and 138.8 mg/dl (SD = 68.65 mg/dl) for TG. We observed a significant increase in total cholesterol levels (+18.43 mg/dl, SD = 23.86 mg/dl, p < 0.0001) and LDL levels (+13.75 mg/dl, SD = 23.05 mg/dl, p = 0.001) in first 12 months after switching from TDF-containing to TAF-containing regimen (**Figure 2**). Changes in both HDL and TG levels were not statistically significant.

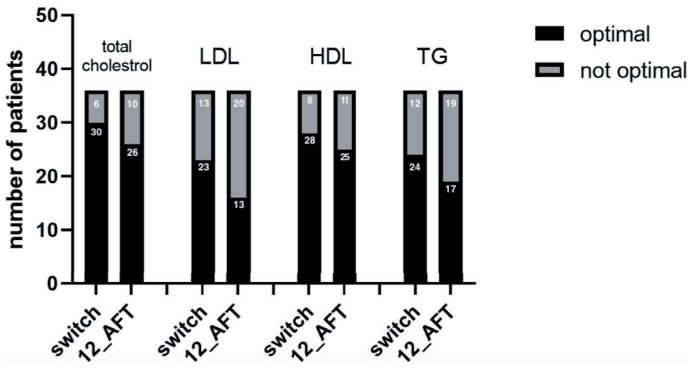

Figure 1. Number of patients with optimal and non-optimal lipid parameters at the time of the switch of ART regimen and 12 months after the switch.

Table 1. Descriptive baseline characteristics for the study population.

	Study group (n = 36)
Age in years, median (IQR)	42 (13.25)
Male, n (%)	32 (87.50)
Female, n (%)	4 (12.50)
Caucasian, n (%)	36 (100.0)
Height (cm), median (IQR)	178 (6.50)
Weight at the time of switching TDF to TAF (kg), median (IQR)	75 (10.75)
BMI at the time of switching TDF to TAF in kg/m^2, median (IQR)	24.20 (3.90)
Duration of HIV infection in years, median (IQR)	9 (5.00)
Duration of HIV treatment in years, median (IQR)	9 (4.50)
Number of patients that in the past had two different ART regimens before switching to a TAF-based regimen, n (%)	14 (38.90)
Number of patients that in the past had three or more different ART regimens before switching to a TAF-based regimen, n (%)	22 (61.10)
Declared route of infection	
– MSM, n (%)	18 (50.0)
– HET, n (%)	12 (33.30)
– IDU, n (%)	2 (5.60)
– no information, n (%)	4 (11.10)
– Co-infection with other STDs (HCV, HBV, syphilis), n (%)	16 (44.40)
Viral load at the time of HIV infection diagnosis (HIV RNA copies/mL), median (IQR)	100892 (368443.00)
CD4+ count at the time of HIV infection diagnosis, median (IQR)	273(183.75)
CD4+ count at the time of the switch to a TAF-based regimen, median (IQR)	585 (346.00)
Cardiovascular comorbidities	
– Hypertension (%)	8 (22.2)
– Heart failure (%)	0 (0)
– Coronary artery disease (%)	0 (0)
– Heart failure (%)	0 (0)
– Previous myocardial infarction (%)	0 (0)
– Previous stroke (%)	0 (0)
– Diabetes mellitus (%)	0 (0)
Other drugs used	
– Statins (%)	9 (25)
– Fibrate (%)	2 (5.6)
– Acetylosalicylic acid (%)	1 (2.8)
– Vitamin K antagonists/novel oral anticoagulants (%)	0 (0)
– Beta-blocker (%)	2 (5.6)
– Calcium channels blocker (%)	3 (8.3)
– ACE-inhibitor/sartan (%)	8 (22.2)
– Diuretics (%)	2 (5.6)
– Hypoglycemic drugs (%)	0 (0)

Legend: HET – heterosexual transmission; MSM – transmission between men having sex with men; IDU – transmission through intravenous drug use; IQR – interquartile range.

During the study period, which is the first year of TAF treatment, there were eight patients treated with statin, one treated with fibrate and one on dual hypolipidemic treatment with statin and fibrate. No changes in the type of hypolipemic drug, dosage or the proportion of patients treated with hypolipemic drugs were observed during the study period.

Next, we investigated whether total cholesterol and LDL levels in individual subgroups of the study group changed regarding patients' baseline BMI, age, and ART duration. A significant increase in total cholesterol and LDL serum levels after switching to a TAF-containing regimen occurred in patients with a BMI below 25 and patients younger than 40 years old. In both sub-

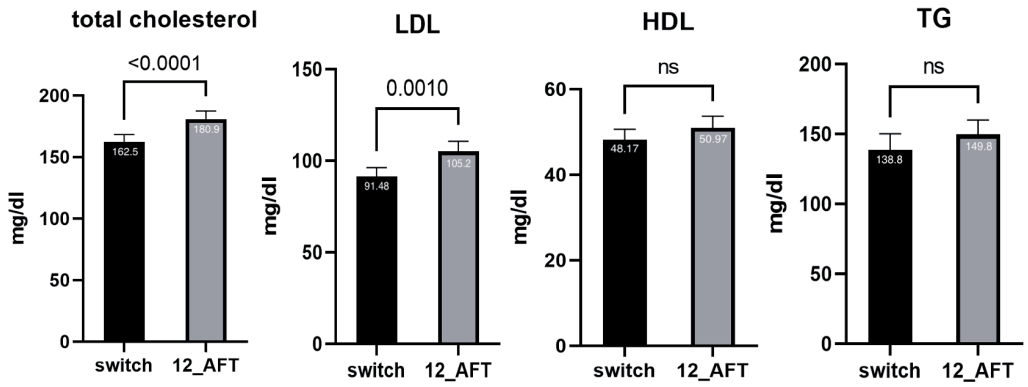

Figure 2. Mean lipid parameter levels at the time of the switch from TDF-containing to TAF-containing regimen and after 12 months of treatment with TAF-containing regimen.

Table 2. Total cholesterol and LDL changes in the study group according to BMI, age, and ART duration.

		Total cholesterol		LDL	
	Number of patients	Mean change (SD) mg/dl	p-value	Mean change (SD) mg/dl	p-value
BMI value					
<25	25	21.27 (23.59)	0.002	18.17 (23.59)	0.0008
≥25	11	11.98 (23.98)	0.13	3.8 (19.34)	0.5125
Age					
<40 years old	22	20.88 (17.79)	<0.0001	12.76 (16.24)	0.0014
≥40 years old	14	14.3 (31.32)	0.11	15.08 (31.71)	0.09
ART duration					
<10 years	21	19.72 (27.45)	0.004	13.53 (24.36)	0.0211
≥10 years	15	17.01 (18.17)	0.003	13.92 (21.66)	0.0233

groups of patients with ART duration of less than or more than ten years, we reported a significant increase in total cholesterol and LDL serum levels (**Table 2**).

Discussion

In the present study describing the effects of a switch from TDF-based to TAF-based ART regimen in a real-world setting, we have observed a significant worsening of lipid parameters among ART-experienced patients in the first year after the switch. The most prominent unfavourable changes in lipid parameters were in total cholesterol (+18.4 mg/dl) and LDL (+13.72 mg/dl) levels. At the same time, there was no significant increase in HDL and TG levels during the study period. Surprisingly, the analysis showed that the effects of regimen change leading to statistically significant worsening of total cholesterol and LDL levels occur mainly among younger patients (below 40 years old) and patients with normal baseline BMI. The study group included in this study consisted of relatively young patients (median age 42), explaining the low rate of cardiovascular comorbidities observed. Nevertheless, we have observed a high rate of patients without optimal lipid parameters after one year after the change of ART regimen and just before the switch. These observations bring concern about the inadequate treatment of lipid disorders or underdiagnosing of dyslipidemias among PLWH. There is an urgent and unfulfilled need for an active search for metabolic disorders in that group of patients. Presented data may help identify groups of patients at a higher risk of developing metabolic disturbances after switching from TDF to TAF, among whom additional surveillance on lipid parameters should be performed.

Since the beginning of the global HIV/AIDS pandemic, which has already resulted in nearly 40 million deaths, the possibilities of treatment for patients infected with HIV have significantly

widened. Currently, long-term treatment with safe antiretroviral drugs provides suppression of HIV replication and reduces its transmission. Regarding the high efficacy of that treatment, prevention of ART-related adverse effects and maintenance of adherence are the main pitfalls that medical professionals need to face. In the era of widely available ART, the population of HIV-positive patients is ageing, which leads to an overlap of HIV-associated as well as age-associated health problems, including neurodegenerative disorders, malignancies, and cardiovascular events [15].

Compared to uninfected patients, PLWH were proven to have a 1.5–2 times higher risk of cardiovascular events, including myocardial infarction, ischemic stroke, heart failure or venous thrombosis [16]. It is the consequence of the interplay of traditional risk factors (such as the high rate of cigarette smokers), chronic viral infection triggering inflammation, and adverse metabolic effects of ART components [18]. Chronic inflammation resulting from the hyperactivity of T cells, macrophages, monocytes and dendritic cells producing excess cytokines causes damage to the endothelium [19]. That exact process results in the formation of necrotic tissue with a mass of foam cells containing LDL, known as atherosclerotic plaques – a morphological manifestation of atherosclerotic disease. High levels of LDL and non-HDL cholesterol were associated with an increase in cardiovascular mortality in the general population, which should be considered when choosing ART components [20]. The worsening of lipids profile after switching ART components brings concerns about the potential atherogenic effect of certain drugs. This phenomenon is especially interesting when TDF is replaced with its newer generic formulation TAF. **Supplementary Table 1** depicts available data from research papers regarding TAF-associated dyslipidemia after the switch from TDF-containing ART.

The treatment with TAF/FTC/EVG/c compared to TDF/FTC/EVG/c results in a higher increase in total cholesterol, LDL, HDL, and TG in 48 weeks among ART-experienced patients [21]. Also, in a phase-3-clinical trial evaluating the safety of switching from RPV/FTC/TDF to RPV/FTC/TAF, researchers reported a significant increase in total cholesterol, LDL, HDL, and TG after 96 weeks among patients who switched from TDF to TAF compared to those who remained on TDF-containing ART [22]. Similar observations to our results were made by research groups from Finland and Ireland that reported the worsening of lipid parameters after switching from TDF-based to TAF-based ART [23,24]. The most significant change was in the total cholesterol and LDL classes. While patients' HDL levels increased only slightly, they were still statistically significant. Another TDF-to-TAF switch study showed nearly the same extent of lipid parameter changes in the time observation during six months. Thus, longer use of TAF in the ART regimen does not lead to more severe dyslipidemia in further months of treatment [25]. This hypothesis is consistent with data obtained by Huhn et al. [26], where patients` dynamics of lipid changes occurred mainly in the first 48 weeks after the ART switch, with only minimal changes from 48 to 96 weeks of observation after initiating HIV infection treatment. After the switch, total cholesterol and LDL levels worsen primarily in patients without baseline hypercholesterolemia [27]. In our group, therefore, we noticed significant changes in the mentioned parameters among patients under 40 years of age and with a normal BMI. However, switching from TDF to TAF in patients with baseline hypercholesterolemia resulted in a significant decrease in LDL/HDL and TC/HDL ratios, markers of ischemic heart disease risk [28]. Interestingly, the effect of TAF on lipids by switching from TDF to TAF is reversible when setting the patient back on TDF-containing ART [29]. That confirms reports about the lipid-lowering properties of TDF on all lipid fractions that may be associated with plasma levels of TFV [30]. Although the lipid-lowering effect of TDF seems to be comparable to some statins, it is still unknown whether the changes in lipid parameters in such cases correspond with a reduction of death risk from cardiovascular events in the future as it was proven to be associated with statin use [31]. It should be stressed that the exact mechanism of TDF improving lipid parameters is unclear. It probably involves actions other than the suppression of HIV replication since this phenomenon was also observed in treating HBV infection [32] and HIV pre-exposure prophylaxis [33].

Both our and other researchers' findings indicate the unfulfilled need for screening for dyslipidemia and assessing the cardiovascular risk of HIV-positive patients treated with ART.

Supplementary Table 1. Current knowledge about the impact of TAF-based ART regimen after switching from TDF-based regimen on lipid parameters among ART-experienced HIV+ patients.

		Median or mean change of lipid parameters (mg/dl)					
No. of patients	Time of observation (weeks)	Total cholesterol	LDL	HDL	TG	Additional information	Study
110	48 weeks on TDF-based ART; 48 weeks on TAF-based ART	+12.50* (median)	+8.20* (median)	+3.00* (median)	+ 4.00 (median)	– presented changes in lipid parameters are between one year before the ART switch and one year after – 13% increase in ASCVD risk scores after switching to TAF	[14]
Included in the analysis: for total cholesterol and TG – 385 for HDL and LDL – 70	12	+20.00* (mean)	+10.00* (mean)	+6.00* (mean)	+23.00* (mean)	– results demonstrate a reversible effect on lipids parameters by switching from TDF to TAF and back	[29]
194	24	+ 14.30* (mean)	+ 9.67* (mean)	+1.90* (mean)	+11.50* (mean)	– the use of statins significantly reduced the risk of worsening lipid panel after switching to TAF	[24]
189	48	+29.00* (mean)	+20.90* (mean)	+3.30* (mean)	+28.90* (mean)	– presented changes in lipid parameters occurred in the group of patients without any lipid-lowering therapy – it was necessary to prescribe almost twice as many lipid-lowering drugs in a group of patients on TAF-based ART compared with TDF-based ART	[21]
Included in the analysis: for total cholesterol – 431 for LDL – 423 for HDL – 426 for TG – 430	12 (median)	+15.00* (mean)	+9.00* (mean)	+5.00* (mean)	+12.00* (median)	– TC, HDL and LDL increased after the switch in patients without HC, while in HC patients, there were no significant variations in TC and LDL, but with a decrease in TC/HDL and LDL/HDL ratio and an increase in HDL	[27]
221	34	+19.00–34.00* (median) depending on other ART agents	+14.00–25.00 (median) depending on other ART agents	+4.00–7.00 (median) depending on other ART agents	+7.00–21.00 (median) depending on other ART agents	– after switching from TDF to TAF, the proportion of patients with LDL above their CV target increased significantly	[39]
347	24	+21.00* (mean)	+14.00* (mean)	+7.00* (mean)	+16.00* (mean)	– despite an increase in total cholesterol, triglycerides and LDL cholesterol after the TDF-to-TAF switch, no difference was found in the LDL:HDL cholesterol ratio, an essential predictor of cardiovascular risk	[25]
490	42	+23.20* (median)	+15.50* (median)	+3.50* (median)	+15.50* (median)	– the increases in lipid concentrations were similar between the participants receiving a non-NRTI, protease inhibitor or INSTI-based ART. Using a boosting agent (ritonavir or cobicistat) did not affect the observed changes in lipid concentrations.	[23]
148	24	+13.40* (mean)	+7.60* (mean)	+3.80* (mean)	+3.00* (median)	– changes in blood lipids did not determine a significant variation in cardiovascular risk scores after six months from the switch	[40]
4328	17 (median)	+12.00* (median)	+8.00* (median)	+2.00* (median)	+14.00* (median)	– 59% of patients with an elevated ASCVD risk were not prescribed statins at any point on or after their first lipid panel after switch	[41]
Included in the analysis: for total cholesterol – 98 for LDL – 95 for HDL – 96 for TG – 98	9 months-2.5 years	+8.70* (mean)	+1.70 (mean)	+2.90* (mean)	+20.00* (mean)	– -study presents underutility of statins after switching from TDF to TAF	[42]
118	52 weeks	no data	+16.00* (median)	no data	+28.00* (median)	– -TAF-based therapy had a statistically significantly worse effect on lipid parameters than TDF- based therapy	[43]

* statistically significant change
ASCVD – atherosclerotic cardiovascular disease

A high proportion of patients with dyslipidemia bring concern about the insufficient administration of lipid-lowering agents in the HIV-positive population. In recent years, the problem of the so-called "statin gap" has been addressed in multiple research papers, indicating that PLWH is less likely to be treated with statins according to current lipid-lowering treatment recommendations compared with HIV-negative patients [34]. What is more, the intensity of treatment has been reported to be inadequate in the context of choosing the type of statin and its daily dosage [35, 36], which may be the result of physicians' fear of potential drug-drug interactions. The metabolism of some statins and ART drugs includes the influence on cytochrome P450, which results in a higher (but still low) risk of drug toxicity and attenuation of the effect of therapy [37]. Nevertheless, the administration of lipid-lowering therapy should be considered a milestone in the long-term care of HIV-positive patients. Potential interactions with antiretrovirals can be managed by careful selection of the appropriate statin or another drug [38]. Also, other factors contributing to non-optimal lipid parameters among patients should be considered, such as non-adherence, suboptimal physician-provider/patient relationships, or overestimating the effect of diet control.

It is essential to mention that this study has some limitations that need to be addressed. Firstly, the sample size was relatively small, with no comparator group that would consist of patients continuing TDF-based treatment. The study's disadvantage is the need for more data about other factors influencing lipid parameters, such as dietary habits or physical activity. Nevertheless, our research provides data from a real-world setting with a comparatively long observation period of one year, which still needs to be discovered among currently published articles.

In conclusion, the presented data suggest that TAF could worsen the lipids parameters in ART-experienced patients, especially those younger than 40 and within the normal BMI range. This effect should be taken into consideration by both clinicians and patients when deciding to include TAF in ART. All PLWHs should be informed of the need to monitor their lipid parameters to facilitate their detection and management of dyslipidemia. Prospective studies on the possible mechanisms behind this metabolic phenomenon, including identifying the risk factors of lipid disorders after switching to a TAF-containing regimen, are required. Professional societies should emphasise the importance of improving the quality of cardiovascular care among PLWHs through early detection and proper management of dyslipidemias.

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Conflict of interest statement
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ORIGINAL PAPER

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SARS-CoV-2 vaccine hesitancy in patients with heart failure: relationship with patient characteristics and pre-pandemic quality of life – a cohort study

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ABSTRACT

Heart failure (HF) patients are vulnerable to a complicated course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. This research analysed the relationship between the decision not to be

immunised against SARS-CoV-2, clinical and epidemiological factors, and the pre-pandemic health-related quality of life (HRQoL) of HF patients. Before the onset of the SARS-CoV-2 pandemic, hospitalised HF patients were enrolled as a prospective cohort and interviewed using the World Health Organization's Quality of Life Brief Version questionnaire. On October 30, 2021, the immunisation status was verified. The association of vaccination hesitancy with epidemiological and clinical parameters and pre-pandemic questionnaire results was tested. Subsequently, independence from confounding factors such as age, sex, the New York Heart Association (NYHA) scale, and left ventricular ejection fraction (LVEF) was analyzed. Among the 136 included patients, 77.9% were vaccinated. Unvaccinated patients were younger (51.2 ± 13.2 vs 56.6 ± 10.3; p = 0.018) and more frequently had non-ischaemic aetiology of HF (73.3% vs 46.7%; p = 0.013). It was significant after adjustment for age, sex, NYHA class, and LVEF. There was no association of overall HRQoL or domain scores with vaccination status. Younger age as a factor associated with vaccine avoidance in this population is consistent with data from the general population despite higher exposure to the severe course of the disease.

Introduction

In December 2019, a new virus called severe acute respiratory failure coronavirus 2 (SARS-CoV-2) caused a global pandemic. SARS-CoV-2 spreads person-to-person by droplet transmission in aerosols [1,2]. Although most people infected with SARS-CoV-2 exhibit mild or moderate respiratory symptoms, some groups are at heightened risk of experiencing a more severe disease course and requiring medical attention [3]. The risk of severe COVID-19 is higher in elderly individuals and those with underlying health conditions.. Patients with HF are also prone to suffer from severe symptoms of COVID-19 [3].

The increase in COVID-19 cases resulted in the intensification of research related to COVID-19 treatment; moreover, vaccines were developed. The European Medicines Agency recommended the first vaccination on December 21 2020, changing the frequency and severity of symptoms and the number of deaths from COVID-19 [4]. Hence, the continuation of vaccination is essential in protecting people from risks associated with COVID-19 disease [5]. Despite clear and substantiated advantages of immunity gained with vaccinations, social movements deny the need to be artificially immunized. During the first pandemic, health professionals faced the challenge of unaware society and widespread perception of infectious diseases as not dangerous, which consequently contributed to questioning the need for vaccination [6–8]. One of the possible reasons could be concerns about vaccine effectiveness and safety [9,10]. However, many studies indicate the benefits and safety of COVID-19 vaccination [11]. The systematic reviews and meta-analysis proved that vaccines have reassuring safety and have an impact on reducing the severe cases, symptomatic cases, and deaths caused by SARS-Cov-2 in a global view, proving its safety [9–11]. The benefits and safety were also evidenced in patients with HF [12]. One of the arguments for refusing to vaccinate in the context of COVID-19 was the short time in which the vaccine was developed and approved by the European Medicines Agency, which was regarded as an experiment on human beings [9]. Among the arguments against vaccination, the theory of the non-existence of a vaccine and saline vaccination and the lack of responsibility of pharmaceutical companies for the side effects were also mentioned [9].

In 2021, the Heart Failure Association of the European Society of Cardiology published a position paper that guides all specialists regarding vaccinations against COVID-19 in patients with HF [13]. Due to that, vaccination is indicated in all patients diagnosed with HF, even those receiving immunosuppressive therapy after heart transplantation or with frailty syndrome. Patients should receive vaccination when in optimal clinical state with the optimized treatment of HF and other comorbidities; however, all corrections should not delay vaccination [13].

It is essential to identify the individuals who refuse SARS-CoV-2 immunization- given the growing problem of vaccine hesitancy. It could help to understand their motivation better and facilitate convincing them to embrace scientifically proven methods of disease prevention. For this study, we aimed to explore the correlation between the characteristics of heart failure patients, their pre-pandemic health-related quality of life, and their decision to decline the SARS-CoV-2 vaccination.

Material and methods

Study population

The HRQoL assessment with the World Health Organization Quality of Life – BREF (WHOQoL-BREF) questionnaire is a standard procedure and was also before the SARS-CoV-2 pandemic outbreak in Poland in patients hospitalized due to HF (the 10th revision of the International Statistical Classification of Diseases and Related Health Problems code for the primary diagnosis). They were enrolled as a prospective cohort from April 2019 to February 2020. After the outbreak of the COVID-19 pandemic and the introduction of vaccines, it was decided to search for an association between clinical and epidemiological factors, pre-pandemic health-related HRQoL and vaccination hesitancy. On October 30, 2021, when every citizen of Poland had the prospect of being immunized against SARS-CoV-2, it was inspected if the patients were vaccinated at least with one dose and if they were alive with the National Health Fund database. Patients who died before October 30, 2021, were excluded. The median time from enrollment to the study to October 30, 2021, was 725 (interquartile range: 480–876) days. Subsequently, the relation of the decision not to be immunized was analyzed in the context of pre-pandemic overall HRQoL and individual HRQoL domains (somatic, psychologic, environmental, and social).

Data collection and questionnaire used

The World Health Organization Quality of Life Brief Version (WHOQoL-BREF) was used to assess the HRQoL of patients with HF. This version has been designed to enable easier and faster assessment of HRQoL. Unlike the WHOQoL instrument, which is based on 100 questions divided into six domains and 24 sub-domains [14], the WHOQoL-BREF consists of 26 questions [15]. The 24 questions are divided into four domains: physical (somatic), psychological, social, and environmental, and there are two additional questions on self-rated HRQoL and satisfaction from health status [15]. It was validated and showed acceptable reliability to substitute the original form [16]. The Polish version of WHOQoL-BREF was used in the research. The acceptable internal consistency was demonstrated with Cronbach's alpha coefficients greater than 0.70 for all domains except the social domain [17]. Questions of the form are listed in Supplementary Materials [18] (**Supplementary Table S1**).

Statistical analysis

According to their distribution, continuous variables are presented as mean ± standard deviation (SD) or median and interquartile range (25th percentile of the data – 75th percentile). Categorical variables are featured as numbers of cases and corresponding percentages in brackets. The Kolmogorov-Smirnov test was used to verify normal distribution. U Mann-Whitney or t-Student's tests were calculated to confront continuous variables according to normality and variance compliance. Pearson's chi-square was used for categorical factors (Yates correction was applied when appropriate). Logistic regression univariable models were counted to define the association of vaccination status with HRQoL, its domains and selected factors (age, sex, NYHA class, LVEF). Predictors of vaccine hesitancy significant or nearly significant ($p < 0.10$) were adjusted for age, sex, NYHA class, and LVEF using logistic regression. The lack of multicollinearity was verified. The secondary analysis then compared unvaccinated patients with an appropriate control group similar in age, sex , disease severity, and comorbidities . A propensity score matching was used to select two control cases for each unvaccinated patient from the remaining 106 patients with the closest neighbourhood method. The propensity score was calculated using logistic regression, considering age, sex, NYHA class III or IV, and diagnosis of diabetes mellitus and chronic kidney disease. A p-value < 0.05 was recognized as significant. Statistical analyses were performed with STATISTICA 13.3 and its Plus Package Tibco Software Inc., Palo Alto, CA, USA.

Ethical statements

This research was approved by the bioethics committee at the Poznan University of Medical Sciences, Poland (no. of approval: 370/20). All human participants gave informed consent to the work.

Results

General characteristics

One hundred thirty-six patients were included in the analysis. The mean age was 55.5 ± 11.2 years, 22.8% were women, mean LVEF was 27.1 ± 11.0% (**Table 1**). Most patients were NYHA class II and III (41.9% and 43.4%, respectively). Concomitant dis-

eases were quite frequent: 53.7% suffered from hypertension, 39.7% had atrial fibrillation or atrial flutter, and 22.8% diabetes mellitus, chronic kidney disease, and chronic obstructive pulmonary disease (COPD) were less frequent (14.7% and 5.9%, respectively). Patients were treated according to the European Society of Cardiology (ESC) guidelines [19]: most of the patients received loop

Table 1. General characteristics of all patients involved in analysis (n = 136). Comparison of basic pre-pandemic parameters between patients who decided to vaccinate against SARS-CoV-2 (n = 106) and those who undertook contrary decision (n = 30).

Parameter	Whole study sample (n = 136)	Unvaccinated group (n = 30)	Vaccinated group (n = 106)	p
Age [years]	55.5 ± 11.2	51.2 ± 13.2	56.6 ± 10.3	0.018
Women	31 (22.8%)	10 (33.3%)	21 (19.8%)	0.14
BMI [kg/m^2]	28.6 ± 5.5	28.4 ± 7.3	28.7 ± 4.9	0.80
non-IHD etiology	72 (52.9%)	22 (73.3%)	56 (46.7%)	0.013
SBP on admission [mmHg]	114.6 ± 19.9	115.8 ± 25.1	114.2 ± 18.2	0.69
DBP on admission [mmHg]	74 (70–80)	71.5 (68–80)	74 (70–80)	0.99
HR on discharge [beats per minute]	73.5 ± 12.1	77.1 ± 9.3	72.4 ± 12.6	0.068
LVEF [%]	25 (20–35)	20 (20–35)	25 (20–35)	0.41
Comorbidities				
DM	31 (22.8%)	5 (16.7%)	26 (24.8%)	0.46
COPD	8 (5.9%)	2 (6.7%)	6 (5.7%)	1.00
CKD	20 (14.7%)	5 (16.7%)	14 (13.3%)	0.77
Hypertension	73 (53.7%)	14 (46.7%)	59 (55.7%)	0.41
AF	54 (39.7%)	9 (30%)	45 (42.5%)	0.29
NYHA class				
I	5 (3.7%)	0	5 (4.7%)	0.29
II	57 (41.9%)	10 (33.3%)	49 (46.2%)	
III	59 (43.4%)	17 (56.7%)	43 (40.6%)	
IV	12 (8.8%)	3 (10%)	9 (8.5%)	
NYHA class III or IV	71 (52.2%)	20 (66.7%)	52 (49.1%)	0.10
Biochemical parameters				
BNP [pg/ml]	398.3 (162.9–802.3)	407.5 (140.2–770.6)	374.9 (184.1–803.65)	0.91
NT proBNP [pg/ml]	1613.5 (590–3042)	1987 (753–4449)	1604 (439–2863)	0.48
Creatinine [µmol/L]	94.4 (79–109.9)	94.5 (75.5–110.1)	94.4 (79.1–109.0)	0.71
eGFR MDRD [mL/min]	75.0 ± 23.9	74.4 ± 25.9	75.1 ± 23.4	0.89
TSH [mIU/L]	1.72 (0.97–3.12)	2.27 (1.38–2.92)	1.46 (0.93–3.12)	0.31
HRQoL				
General HRQoL (0–400)	265.6 (237.1–288.3)	264.0 (240.9–307.9)	265.7 (236.6–288.2)	0.99
Somatic domain (transformed score 0–100)	53.6 (46.4–57.1)	50 (39.3–57.1)	53.6 (46.4–57.1)	0.22
Psychological domain (transformed score 0–100)	66.7 (58.3–70.8)	70.8 (58.3–79.2)	62.5 (58.3–70.8)	0.12
Social domain (transformed score 0–100)	75 (66.7–91.7)	75 (66.7–91.7)	75 (66.7–91.7)	0.86
Environmental domain (transformed score 0–100)	71.9 (62.5–81.2)	73.4 (59.4–78.1)	71.9 (62.5–81.2)	0.88
Medications				
Loop diuretics [%]	124 (91.2%)	30 (100%)	94 (88.7%)	0.068
Thiazides [%]	19 (14.0%)	7 (23.3%)	12 (11.3%)	0.13
β-blocker [%]	131 (96.3%)	30 (100%)	101 (95.3%)	0.59
ACEI/ARB [%]	89 (65.4%)	20 (66.7%)	69 (65.1%)	1.00
ARNI [%]	33 (24.3%)	6 (20%)	27 (25.5%)	0.63
MRA [%]	115 (84.6%)	24 (80%)	91 (85.9%)	0.41
Ca-blocker [%]	9 (6.6%)	2 (6.7%)	7 (6.6%)	1.00
Statin [%]	90 (66.2%)	15 (50%)	75 (70.8%)	0.048

Abbreviations: BMI – body mass index, IHD – ischaemic heart disease, SBP – systolic blood pressure, DBP – diastolic blood pressure, HR– heart rate, LVEF –left ventricular ejection fraction, COPD – chronic obstructive pulmonary disease, CKD – chronic kidney disease, AF – atrial fibrillation (paroxysmal, permanent or persistent), NYHA – New York Heart Association Classification, BNP – B-type natriuretic peptide, NT proBNP – N-terminal pro-B-type natriuretic peptide, eGFR – estimated glomerular filtration rate, MDRD – Modification of Diet in Renal Disease, TSH – thyroid stimulating hormone, HRQoL —health-related quality of life, ACEI – angiotensin-converting enzyme inhibitor, ARB – angiotensin receptor blocker, ARNI – angiotensin receptor-neprilysin inhibitor, MRA—mineralocorticoid receptor antagonist.

diuretics, beta-blockers, angiotensin-converting enzyme inhibitor ACEIs or angiotensin receptor blockers (ARBs) or angiotensin receptor-neprilysin inhibitor (ARNI), and mineralocorticoid receptor antagonist (MRA). As of the 30th of October 2021, 77.9% of patients had been vaccinated.

Comparison of basic parameters between vaccinated and unvaccinated

Patients who decided not to vaccinate against SARS-CoV2 in the follow-up period were significantly younger and more frequently had non-IHD aetiology (**Table 1**). Cohesively with differences in aetiology, they less frequently had statin prescribed. The relation of overall HRQoL or respective domains according to WHOQoL-BREF and immunization status was not demonstrated (**Table 2**). In multivariable analysis, younger age, independent of sex, NYHA class, and LVEF, is significantly associated with not vaccinating in the logistic regression multivariable model (OR 0.950 95% CI 0.913–0,990; p = 0.014) (**Table 2**). Similarly, non-ischaemic aetiology of HF was related to vaccine hesitancy independent of age, sex, NYHA class, and LVEF (OR 0.559 95% CI 0.334–0.935; p = 0.027).

Comparison of unvaccinated patients and matched control group

The control group was matched for age, sex, NYHA class III or IV, and diagnosis of diabetes mellitus and chronic kidney disease (**Supplementary materials**, **Supplementary Table S2**). It was not significantly different from unvaccinated patients regarding overall HRQoL and any of its domains.

Discussion

The SARS-CoV-2 pandemic changed the world, including daily routines, businesses, and people's well-being. In this study, we examine the relationship between the characteristics of the HF patients, pre-pandemic HRQoL, and their resignation from vaccination against SARS-CoV-2. Understanding the reasons for the lack of vaccination acceptance is crucial for better clinician-patient cooperation. The younger age of the HF patients was a predictor of non-vaccination, independent of sex, NYHA class, and LVEF. It has yet to be studied in a distinct group of patients with heart failure. However, there are numerous reports that in the general population, younger age is associated with vaccine hesitancy [10–12,20–22], as well as in the Polish population [23,24]. There are a few possible causes for this. Firstly, they could feel less at risk of severe infection [25–28] – the risk increases with older age, which is well known [10,29–32]. However, despite their relatively young age, heart failure patients are also at risk of severe course of the disease. Therefore, there may be another explanation for their hesitancy towards vaccination.

On the other hand, there were concerns about the impact of the vaccine on future fertility [33]. Women in the perinatal period were

Table 2. Logistic regression results: univariable and multivariable after adjustment for age, sex, NYHA class, and LVEF.

Variable	Univariable OR 95%CI	p- value	Adjusted* OR 95%CI	p- value
Age (years)	0.959 (0.925–0.995)	0.026	0.950 (0.913–0.990)	0.014
Male sex	0.525 (0.215–1.281)	0.16	-	-
NYHA III/IV	1.694 (0.734–3.912)	0.22	-	-
LVEF (%)	0.994 (0.958–1.032)	0.75	-	
Non-ischaemic aetiology	0.564 (0.360–0.883)	0.012	0.559 (0.334–0.935)	0.027
Total HRQoL	0.997 (0.988–1.007)	0.60	-	-
Somatic D	0.902 (0.789–1.031)	0.13	-	-
Psychological D	1.106 (0.948–1.291)	0.20	-	-
Social D	0.925 (0.776–1.103)	0.39	-	-
Environmental D	0.988 (0.900–1.084)	0.80	-	-

* Variables significant or nearly significant in univariate analysis (with p < 0.10) were adjusted for age, sex, NYHA class, and LVEF.

Abbreviations: NYHA – New York Heart Association Classification, LVEF – left ventricular ejection fraction, HRQoL – health-related quality of life, D – domain, OR – the odds ratio (with 95% confidence interval), CI – confidence interval, p-value – probability value, indicate significant values (p < 0.05).

reluctant to vaccinate against COVID-19 due to fears about the vaccine's safety [34]. Moreover, the general belief in conspiracy theories among younger people is more abundant than in older ones [35]. Another factor could be the higher risk of myocarditis following vaccines [36], which is most prominent in younger men after the second dose of the messenger ribonucleic acid vaccine [37]. However, studies have shown that overall, the risk of myocarditis is higher after SARS-CoV-2 infection than after SARS-CoV-2 vaccination [38]. The latter may also have been the reason for higher vaccination hesitancy in the non-ischaemic aetiology of HF, as myocarditis is one of the triggers for chronic HF, and patients could be afraid of its recurrence.

This study found no differences in pre-pandemic HRQoL between vaccinated and unvaccinated patients. Minimal data on the relation between HRQoL and vaccination status were found, with no studies on HF patients. One study involved a sample of almost 30,000 Chinese in the general population [10]. Authors reported that HRQoL measured with the EQ-5D instrument was worse in the unvaccinated population [10]. In our study, we observed no significant differences. However, there are numerous differences in the study population and design. Several studies have examined the relationship between HRQoL and vaccination decisions, but none have focused on patients with heart failure [26,39–41]. Lin et al. [39], in a subgroup of general population participants aged below 50, reported significantly worse physical HRQoL in the unvaccinated people than in the vaccinated subjects. Wu et al. [40] examined heart transplant recipients with the 36-item Short Form Survey. Patients vaccinated against COVID-19 had better physical and mental components of the survey results [40]. Nguyen et al. [26] related worse HRQoL measured in lower educational attainment and lower income with COVID-19 vaccine hesitancy in a representative sample of adults in the United States. Babicki et al. [41] revealed better scores in the Manchester Short Assessment of Quality of Life survey and lower levels of anxiety measured by the Generalized Anxiety Disorder Assessment in a vaccinated sample of the general population compared to non-immunized.

This study's results are valuable because data were gathered with face-to-face interviews with consecutive hospitalised patients, providing better data quality than online surveys used in most of the mentioned studies [10,25,26,41].

When it comes to sex differences, many studies, including meta-analyses based on numerous papers, have shown that women are less willing to vaccinate [10,24,42,43]. At the same time, other reports have found no significant differences [21,44–46] or, on the contrary, found that men are more hesitant to get vaccinated [22,47]. These sex differences may be related to cultural and socioeconomic differences. In a recent study on the Polish population, based on online surveys of young adults (mean ages: 22.8 and 31.2 years compared to 55.5 years in this article), men were less likely to avoid vaccination. Considering the available meta-analysis [42], most of the analysed studies were surveys, often conducted online, that included much younger patients, especially those working in the healthcare or government sector, and asked about attitudes towards vaccinations rather than verifying actual decisions. The study by Williams et al. revealed no significant sex differences in the population of vulnerable to the severe course of COVID-19 patients – those aged above 65 years and younger patients with chronic respiratory disease [46]. This study is based on registry data, analyzing older patients with chronic disease with a poor prognosis. Therefore, extrapolating data from cited works on this population is unjustified. On the other hand, the lack of statistically significant sex differences in the results may be related to population size, so analysis of sex differences in vulnerable HF patients would require the inclusion of a larger population.

Study limitations

The article provides only a limited view of the unvaccinated patients with HF, and the results should not be generalized. However, the analyzed population was homogenous, including only HF patients prone to severe course of COVID-19. The group of non-immunized constituted a minority, which resulted in a relatively low number of unvaccinated patients in the study. Unvaccinated people should not be equalized as anti-vaccines; they were not asked to explain their motivation and did not check if some of them had permanent contraindica-

tions to vaccination with available specimens (although they are infrequent, including anaphylactic reactions to the vaccine or its ingredients [48]). However, such an approach allowed to avoid non-response bias due to resignation from the study of some unvaccinated people. Patient comorbidities and other unrecognized factors could influence HRQoL. To reduce this limitation, we used multivariable analysis and a control group matched with propensity score (**Supplementary materials: Table S2**) . Propensity score matching itself has some significant limitations. Firstly, the method may need to include all clinically essential factors that can influence the results.

Moreover, there are usually still non-significant differences between both groups regarding factors included in score counting. Furthermore, it reduces the size of the sample, decreasing statistical significance. At last, patients were assessed about two years before the pandemic had begun, and their clinical status could have deteriorated, and HRQoL could have changed. However, age differences and the aetiology of HF are stable over time.

Conclusions

Younger HF patients are more hesitant to vaccinate for COVID-19. Moreover, non-ischaemic aetiology is associated with resignation from vaccination independently from age, sex, NYHA class, and LVEF. The study found no significant association between pre-pandemic overall HRQoL, its domains and vaccination status. The group most prone to COVID-19 vaccine hesitancy in HF are relatively young patients with non-ischaemic aetiology of HF.

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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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Lower uric acid and adequate hydration are associated with lower risk of febrile neutropenia following autologous bone marrow transplantation in patients with lymphoma

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ABSTRACT

Background. Despite the promising results of autologous bone marrow transplantation (BMT) in patients with lymphoma, infectious complications limit its positive outcomes. This study evaluated the incidence and associated factors of febrile neutropenia (FN) following BMT in patients with lymphoma.

Material and methods. The study consecutively included 147 patients with lymphoma who were candidates for BMT. Clinical and laboratory results were recorded, and after BMT, the occurrence of FN was investigated through the daily evaluation of neutrophil count and body temperature.

Results. On average, FN occurred in 91 patients (61.9%) after 12.77 ± 2.45 days after BMT. Lower fluid balance was associated with a higher risk of FN (lowest adjusted odds ratio [OR] at day -2 = 0.602, 95% confidence interval [CI] = 0.299 – 0.870, p-value = 0.007). The higher uric acid level was associated with a higher risk of FN (highest adjusted OR at day -10 = 1.617, 95% CI = 1.328 – 1.963, p-value = 0.035). LDH was also positively correlated with FN (highest adjusted OR at day 0 = 1.501, 95% CI = 1.198 – 2.104, p-value = 0.004).

Conclusions. Adequate hydration of the patients is of paramount importance for preventing FN in patients who receive BMT. Furthermore, uric acid and LDH could be considered in future studies for the risk stratification of FN.

Introduction

Lymphomas are classified into two major categories: non-Hodgkin (90%, NHL) and Hodgkin (10%, HL) lymphomas [1]. The global incidence of HL has increased by 38.66%, from 72,937 in 1990 to 101,133 in 2017; however, due to improved treatment modalities, the age-standardized death rate and the annual age-standardized disability-adjusted life year (DALY) have decreased during these years [2,3]. After high-dose chemotherapy, bone marrow transplantation (BMT) or autologous hematopoietic stem cell transplantation (AHSCT) is the standard way to treat lymphomas that do not respond to treatment or have come back [4]. Patients with high-risk aggressive lymphoma who are unresponsive to conventional regimens alone show a better prognosis by receiving early consolidative transplantation. Moreover, it can significantly diminish the bone marrow tumour load and consequently bring about a higher complete remission rate [4]. Despite substantial positive advances due to these therapeutic modalities, transplant-related infectious complications are still causing considerable morbidity and mortality in lymphoma patients [5]. These complications occur in a varying range of 12 up to 51% of transplanted patients [6,7]. Higher incidences are documented in the neutropenic phase or pre-engraftment [8,9]. Moreover, the incidence of severe sepsis in AHSCT patients was reported to be five times higher than in the non–AHSCT cohort, and it was associated with a mortality rate of 32.9 percent in AHSCT patients [10]. A fever in people with a bone marrow transplant is a sign of bacteremia, often linked to low white blood cells (febrile neutropenia, or FN) [11]. Several associated factors have been suggested for the higher incidence of infectious complications, including > 18 years of age, use of unrelated graft source and myeloablative conditioning regimen, transplant-associated thrombotic microangiopathy, acute graft versus host disease, high-risk malignant disease, mucositis, and steroid administration [12,13]. Nevertheless, the failure to prevent these complications necessitates further investigations into other previously overlooked factors associated with them. Furthermore, detecting these factors could help limit the use of antibacterial prophylaxis to high-risk patients to prevent multi-drug-resistant microorganisms [14,15]. In this study, the incidence and associated factors of occurrence of FN were evaluated in patients with lymphoma who receive BMT after high-dose chemotherapy.

Material and methods

Study design and patients' inclusion

In this prospective study, 147 adult patients (aged between 18 and 65 years) with a definite diagnosis of lymphoma (HL or NHL) who were candidates for BMT due to relapse following standard chemotherapy and admitted to the Ghazi Hospital in Tabriz (the only tertiary referral hospital for haemato-oncological diseases in north-west Iran) were consecutively included between December 1, 2016, and July 30, 2022. Patients were excluded if they had co-morbidities and those who were heavily treated (received more than three chemotherapy protocols). The study was conducted following the declaration of Helsinki, and each patient gave informed consent. We obtained ethical clearance from the medical ethics committee at Tabriz University of Medical Sciences in Iran.

Medical procedure

All patients received standard medical care during hospitalization. Patients were isolated in positive-pressure reverse isolation rooms and were indicated to avoid raw fruits and vegetables. No medication was administered by rectal, vaginal, or intramuscular routes. The hospital staff employed strict handwashing procedures using disinfectant agents before entering patients' rooms. BMT and standard medical care were performed following established guidelines [16]. After admission, patients received a conditioning regimen including lomustine (CCNU) 200 mg/m^2 (one dose), cytarabine 400 mg/m^2 (two times daily for two days), VP16 400 mg/m^2 (two times daily) and melphalan 140 mg/m^2 (once). Later, stem cell collection was conducted using apheresis. The following day, BMT was performed using intravenous infusion of the obtained stem cells. Moreover, all patients received a prophylaxis regimen since the day of admission and also after BMT, including acyclovir 800 mg (two times daily), fluconazole 150 mg (two times daily), and ciprofloxacin 500 mg (two times daily during the neutropenic days). They also received trimethoprim

/ Sulfamethoxazole 800 mg/160 mg (two times daily for two days per week) from the day of BMT until three months later [16].

Study variables

Clinical and paraclinical variables were selected based on our clinical experiences and a preliminary literature review. Demographic characteristics, lymphoma type, and medical history of included patients were recorded in a pre-prepared questionnaire. Laboratory blood tests and fluid balance measurements (Intake–Output) were conducted three, two and one day before transplantation and on the transplantation day. After transplantation, the neutrophil count was measured daily, and body temperature was evaluated meticulously in the armpit (axillary). A neutrophil count less than 1.5×10^9/L was considered neutropenia [17]. Body temperature (BT) was measured at 8–9 AM each day after transplantation, and the patient was considered febrile if they had BT > 38.3° C or BT > 38 if it lasted more than one hour. As elucidated above, FN was defined as a simultaneous presence of fever and neutropenia.

Profound neutropenia was defined as a neutrophil count < 500 cells/μL or a neutrophil count < 1000 cells/μL when a further decrease to < 500 cells/μL over the next 48 hours was expected [18].

Statistical analysis

Data are presented as mean ± standard deviation (SD) or frequency and percentage. The Kolmogorov-Smirnov test evaluated the normal distribution of the data. Chi-square or Fischer's exact test was conducted on categorical data, and the student T-test or Mann-Whitney U test was used to compare groups in parametric and non-parametric data, respectively. Simple and multiple logistic regression analyses were conducted in the context of univariate and multivariate analyses, and unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (CI) were calculated. The analysis was performed using SPSS v 24. A p-value < 0.05 was considered statistically significant.

Results

A total of 147 lymphoma patients were included. **Table 1** describes the baseline characteristics of

Table 1. Baseline characteristics of lymphoma patients undergoing bone marrow transplantation with and without febrile neutropenia.

	Subtype	Total	Febrile neutropenia (n = 91)	Non-Febrile neutropenia (n = 56)	P value
Age $_{\text{mean ± SD}}$		35 (10)	37 (11)	31 (10)	0.113*
BMI $_{\text{mean ± SD}}$		30.05 (22.14)	32.18 (27.84)	26.55 (4.36)	0.512**
Gender $_{\text{n (%)}}$	Male	104 (70.7)	61 (67.0)	43 (76.7)	0.733#
	Female	43 (29.3)	30 (33.0)	13 (23.3)	
Type of lymphoma $_{\text{n (%)}}$	NHL	48 (32.7)	29 (31.9)	19 (33.9)	0.990#
	HD	99 (67.3)	62 (68.1)	37 (66.1)	
Blood group $_{\text{n (%)}}$	A+	37 (25.2)	17 (18.7)	20 (35.3)	0.595$
	A-	5 (3.4)	2 (2.2)	3 (5.9)	
	B+	30 (20.4)	20 (22.0)	10 (17.6)	
	B-	5 (3.4)	5 (5.4)	0 (0)	
	AB+	9 (6.1)	9 (9.9)	0 (0)	
	AB-	0 (0)	0 (0)	0 (0)	
	O+	52 (35.4)	32 (35.2)	20 (35.3)	
	O-	9 (6.1)	6 (6.6)	3 (5.9)	
Number of radiotherapy courses before BMT $_{\text{mean ± SD}}$		11 (12)	10 (12)	19 (5)	0.471**
Number of chemotherapy courses before BMT $_{\text{mean ± SD}}$		14 (5)	15 (6)	12 (4)	0.244**

* Student T-test was used (two-sided significance);** Mann-Whitney U test was used (two-sided significance)# Fisher's exact test was used (two-sided significance) $ Pearson chi-square test was used.
BMI = Body mass index, BMT = Bone marrow transplantation, NHL = Non-Hodgkin lymphoma, HL = Hodgkin lymphoma, SD = Standard deviation, n = Number.

Table 2. Laboratory test results before bone marrow transplantation in lymphoma patients with and without febrile neutropenia.

	Days before BMT	Total	Febrile neutropenia	Non-Febrile neutropenia	P-value
ESR (mm/h)	0	21.41 (14.66)	26.00 (7.31)	14.38 (10.02)	0.002
	-5	23.37 (15.89)	27.48 (16.35)	17.07 (13.32)	0.048
	-10	17.49 (13.32)	21.27 (15.21)	11.71 (6.71)	0.050
CRP (mg/L)	0	1 (1)	1 (1)	1 (1)	0.649
	-5	1 (1)	2 (1)	1 (1)	0.326
	-10	0 (1)	0 (1)	0 (0)	0.506
Uric acid (mg/dL)	0	4.08 (1.36)	4.70 (1.51)	3.74 (1.18)	0.048
	-5	6.38 (2.16)	7.96 (2.25)	5.74 (1.80)	0.012
	-10	4.91 (1.32)	5.58 (1.33)	4.60 (1.22)	0.021
Fibrinogen (mg/dL)	0	336.87 (119.83)	347.41 (137.68)	317.29 (82.87)	0.311
	-5	351.23 (74.98)	365.37 (81.45)	328.60 (64.91)	0.435
	-10	323.91 (109.81)	343.46 (110.55)	298.50 (109.13)	0.446
LDH (IU/L)	0	373 (351)	460 (127)	289 (89)	0.030
	-5	415 (170)	476 (216)	341 (113)	0.006
	-10	484 (184)	600 (180)	362 (104)	0.007
WBC (cells/L)	0	6031 (6328)	4674 (4683)	8187 (7995)	0.094
	-5	29731 (15948)	31702 (15907)	26485 (15952)	0.349
	-10	8490 (7732)	8065 (5593)	9190 (10525)	0.598
N (cells/L)	0	4580 (5795)	3276 (4198)	6652 (7361)	0.151
	-5	25868 (13970)	26474 (14559)	24871 (13315)	0.797
	-10	7689 (7060)	7423 (5249)	8128 (9500)	0.607
Hb (g/dL)	0	11.3 (1.5)	11.0 (1.5)	11.7 (1.6)	0.223
	-5	11.3 (1.3)	11.1 (1.3)	11.7 (1.3)	0.150
	-10	10.8 (1.9)	10.6 (1.2)	11.2 (2.8)	0.129
Plt (cells $*10^3$/L)	0	129.5 (63.2)	126.9 (52.1)	133.6 (79.2)	0.791
	-5	143.2 (60.6)	135.2 (53.6)	156.4 (70.2)	0.367
	-10	104.9 (48.0)	98.85 (48.3)	114.9 (47.2)	0.246
MPXI (Index)	0	7.01 (13.74)	6.72 (15.84)	7.47 (9.94)	0.399
	-5	-2.45 (8.59)	-3.60 (8.50)	-0.56 (8.67)	0.178
	-10	-4.89 (9.39)	-4.89 (9.71)	-4.90 (9.13)	0.935
LUC (109/L)	0	0.31 (0.85)	0.41 (1.07)	0.14 (0.11)	0.276
	-5	0.52 (0.62)	0.56 (0.74)	0.44 (0.32)	0.990
	-10	0.12 (0.14)	0.14 (0.13)	0.10 (0.16)	0.225
Retic count (%)	0	1.07 (0.90)	1.06 (1.13)	1.08 (0.70)	0.720
	-5	1.50 (0.00)	1.50 (0.00)	-	-
	-10	0.35 (0.21)	0.35 (0.21)	-	-
Fluid balance (ml)	-1	771.05 (902.51)	300.00 (53.19)	892.73 (297.29)	0.004
	-2	879.47 (195.00)	563.75 (162.87)	1418.18 (165.1)	0.012
	-3	437.50 (960.99)	148.89 (693.28)	835.45 (991.48)	0.038
Ferritin (ug/L)		518.17 (403.20)	468.38 (462.61)	558.00 (369.68)	0.360
CD34 ($\times 10^6$)		14.56 (49.34)	22.82 (65.11)	3.75 (2.45)	0.837
Number of administered packed cells		1 (1)	2 (1)	1 (1)	0.038**
Number of administered units of platelet		16 (11)	19 (12)	12 (3)	0.014**

Data are presented as mean ± SD; the analysis is performed using the Mann-Whitney U test.
Days are reported concerning the date of bone marrow transplantation (BMT): 0 = the day of BMT, -5 = five days before BMT, -10 = ten days before BMT; Hb = Hemoglobin, Plt = platelet, MPXI = Myeloperoxidase index, LUC = Large unstained cells.

Table 3. Relationship between study variables and febrile neutropenia in lymphoma patients undergoing bone marrow transplantation.

	Subtype/ day	Unadjusted Odds ratio	95% CI		P-value	Adjusted Odds ratio*	95% CI		P-value
			Lower	Upper			Lower	Upper	
Age		1.056	0.986	1.13	0.117	-	-	-	-
Gender	Male	0.650	0.165	2.564	0.538	-	-	-	-
	Female	Ref	-	-	-	-	-	-	-
BMI		1.028	0.938	1.128	0.553	-	-	-	-
Type of lymphoma	NHL	0.868	0.243	3.099	0.828	-	-	-	-
	HL	Ref	-	-	-	-	-	-	-
Number of radiotherapy courses		0.928	0.797	1.081	0.337	-	-	-	-
Number of chemotherapy courses		1.12	0.973	1.29	0.115	-	-	-	-
Number of administered packed cells		1.766	1.01	3.089	0.046	2.022	.917	4.462	0.081
Blood type	A+	Ref	-	-	-	-	-	-	-
	A-	0.500	0.013	19.562	0.711	-	-	-	-
	B+	1.000	0.063	15.988	1.000	-	-	-	-
	B-	-	0.000	-	0.999	-	-	-	-
	AB+	-	0.000	-	0.999	-	-	-	-
	AB-	0.750	0.055	10.233	0.829	-	-	-	-
	O+	0.417	0.013	6.064	0.522	-	-	-	-
	O-	0.500	0.063	19.562	0.711				
Number of administered units of platelet		1.082	1.000	1.173	0.050	2.118	1.010	2.937	0.031
ESR	0	1.034	0.987	1.084	0.061				
	-5	1.057	0.998	1.118	0.057				
	-10	1.078	1.007	1.155	0.032	1.084	.998	1.176	0.056
CRP	0	1.091	0.636	1.87	0.752	-	-	-	-
	-5	1.343	0.774	2.327	0.294	-	-	-	-
	-10	2.263	0.557	9.189	0.253	-	-	-	-
Uric acid	0	1.572	1.318	1.827	0.061	1.601	1.124	2.101	0.007
	-5	1.578	1.368	1.909	0.018	1.525	1.288	1.958	0.036
	-10	1.535	1.300	1.953	0.034	1.617	1.328	1.963	0.035
Fibrinogen	0	1.002	0.994	1.01	0.587	-	-	-	-
	-5	1.008	0.99	1.026	0.385	-	-	-	-
	-10	1.004	0.996	1.013	0.329	-	-	-	-
LDH	0	1.401	1.098	1.904	0.007	1.501	1.198	2.104	0.004
	-5	1.411	1.099	1.941	0.007	1.471	1.118	1.933	0.006
	-10	1.421	1.097	1.955	0.006	1.462	1.107	1.915	0.007
WBC	0	1	1	1	0.087	-	-	-	-
	-5	1	1	1	0.288	-	-	-	-
	-10	1	1	1	0.637	-	-	-	-
N	0	1	1	1	0.072	-	-	-	-
	-5	1	1	1	0.706	-	-	-	-
	-10	1	1	1	0.744	-	-	-	-
Hb	0	0.748	0.493	1.135	0.172	-	-	-	-
	-5	0.69	0.415	1.148	0.153	-	-	-	-
	-10	0.838	0.576	1.221	0.359	-	-	-	-
Plt	0	1	1	1	0.729	-	-	-	-
	-5	1	1	1	0.264	-	-	-	-
	-10	1	1	1	0.276	-	-	-	-
MPXI	0	0.996	0.953	1.041	0.858	-	-	-	-
	-5	0.955	0.882	1.034	0.254	-	-	-	-
	-10	1	0.937	1.067	0.996	-	-	-	-
LUC	0	22.895	0.12	4373.027	0.243	-	-	-	-
	-5	1.446	0.415	5.036	0.562	-	-	-	-
	-10	7.005	0.057	868.347	0.429	-	-	-	-
Fluid balance	-1	0.680	0.331	0.841	0.004	0.670	0.312	0.801	0.004
	-2	0.638	0.399	0.801	0.009	0.602	0.299	0.870	0.007
	-3	0.799	0.398	0.890	0.007	0.637	0.304	0.708	0.006
Ferritin		0.999	0.997	1.002	0.632	-	-	-	-

* Adjusted for age, gender, Body mass index (BMI), and type of lymphoma. Only those significant variables in unadjusted simple regression were considered in multiple regression analysis.
Days are reported in relation to date of BMT (0 = the day of BMT, -5 = five days before BMT, -10 = ten days before BMT), N = neutrophil count, Hb = Hemoglobin, Plt = platelet, MPXI = Myeloperoxidase index, LUC = Large unstained cells.

Table 4. Relationship between study variables and febrile neutropenia with profound neutropenia in lymphoma patients undergoing bone marrow transplantation.

	Subtype/ day	Unadjusted Odds ratio	95% CI		P-value	Adjusted Odds ratio*	95% CI		P-value
			Lower	Upper			Lower	Upper	
Age		1.864	0.613	3.115	0.606	-	-	-	-
Gender	Male	1.729	0.212	3.245	0.539	-	-	-	-
	Female	0.886	0.505	1.267	0.532	-	-	-	-
BMI		1.384	0.030	2.738	0.570	-	-	-	-
Type of lymphoma	NHL	2.938	0.589	5.286	0.593	-	-	-	-
	HL	0.811	0.426	1.196	0.817	-	-	-	-
Number of radiotherapy courses		2.171	0.182	4.160	0.800	-	-	-	-
Number of chemotherapy courses		1.036	0.400	1.671	0.523	-	-	-	-
Number of administered packed cells		1.800	1.735	1.865	0.035	1.913	-1.615	5.441	0.629
Blood type	A+	2.426	0.789	4.062	0.741	-	-	-	-
	A-	1.431	0.711	2.150	0.826	-	-	-	-
	B+	0.690	0.071	1.310	0.600	-	-	-	-
	B-	2.600	0.173	5.027	0.636	-	-	-	-
	AB+	1.045	0.063	2.027	0.834	-	-	-	-
	AB-	1.184	0.817	1.550	0.600	-	-	-	-
	O+	2.765	0.303	5.228	0.957	-	-	-	-
	O-	1.984	0.022	3.946	0.752	-	-	-	-
Number of administered units of platelet		1.600	1.543	1.656	0.025	1.924	1.867	1.981	0.014
ESR	0	2.045	0.876	3.214	0.846	-	-	-	-
	-5	1.602	0.884	2.321	0.197	-	-	-	-
	-10	2.781	0.869	4.693	0.737	-	-	-	-
CRP	0	2.846	0.733	4.960	0.542	-	-	-	-
	-5	2.102	0.608	3.596	0.853	-	-	-	-
	-10	2.486	0.525	4.447	0.987	-	-	-	-
Uric acid	0	1.356	1.224	1.488	0.008	1.748	1.615	1.880	0.010
	-5	1.167	1.121	1.212	0.030	1.606	1.341	1.871	0.034
	-10	1.326	1.060	1.591	0.027	1.654	1.522	1.786	0.031
Fibrinogen	0	0.939	0.645	1.233	0.379	-	-	-	-
	-5	2.843	0.659	5.027	0.796	-	-	-	-
	-10	0.627	0.153	1.101	0.239	-	-	-	-
LDH	0	1.942	1.885	1.998	0.005	1.952	1.687	2.217	0.015
	-5	1.739	1.683	1.796	0.006	1.416	1.370	1.462	0.021
	-10	1.468	1.202	1.733	0.045	1.043	0.987	1.100	0.035
WBC	0	2.926	0.853	4.999	0.846	-	-	-	-
	-5	1.777	0.178	3.376	0.521	-	-	-	-
	-10	1.190	0.462	1.917	0.225	-	-	-	-
N	0	0.510	0.445	0.575	0.124	-	-	-	-
	-5	1.944	0.336	3.551	0.071	-	-	-	-
	-10	2.937	0.530	5.343	0.630	-	-	-	-
Hb	0	0.671	0.646	0.696	0.041	-	-	-	-
	-5	0.668	0.501	0.835	0.038	-	-	-	-
	-10	0.599	0.364	0.833	0.039	-	-	-	-
Plt	0	2.496	0.172	4.819	0.883	-	-	-	-
	-5	2.247	0.383	4.110	0.381	-	-	-	-
	-10	1.213	0.266	2.160	0.720	-	-	-	-
MPXI	0	2.868	0.484	5.252	0.681	-	-	-	-
	-5	1.954	0.351	3.557	0.410	-	-	-	-
	-10	2.169	0.616	3.722	0.963	-	-	-	-
LUC	0	1.467	0.749	2.185	0.110	-	-	-	-
	-5	0.925	0.031	1.819	0.309	-	-	-	-
	-10	0.913	0.573	1.253	0.939	-	-	-	-
Fluid balance	-1	0.344	0.144	0.544	0.015	0.424	0.206	0.643	0.004
	-2	0.531	0.516	0.546	0.024	0.769	0.640	0.898	0.033
	-3	0.639	0.603	0.674	0.038	0.827	0.755	0.899	0.024
Ferritin		1.349	0.606	2.093	0.813	-	-	-	-

* Adjusted for age, gender, Body mass index (BMI), and type of lymphoma. Only those significant variables in unadjusted simple regression were considered in multiple regression analysis.
Days are reported concerning the date of BMT (0 = the day of BMT, -5 = five days before BMT, -10 = ten days before BMT), N = neutrophil count, Hb = Hemoglobin, Plt = platelet, MPXI = Myeloperoxidase index, LUC = Large unstained cells.

the patients. Patients' age was 34.79 ± 10.49, and 29.3% of the patients were female. On average, FN occurred in 91 patients (61.9%) after 12.77 ± 2.45 days after BMT.. No statistically significant difference was seen between the two groups (those with FN and those without) in terms of age, gender, BMI, type of lymphoma, blood group, and the number of radiotherapy or chemotherapy courses ($p > 0.05$ for all, **Table 2**). Patients with FN had received packed cells more frequently than the non-FN group (2 ± 1 in the FN group vs 1 ± 1 in the non-FN group, $p = 0.038$, **Table 3**). Also, more platelet units were administered in the FN group (19 ± 12 in the FN group vs 12 ± 3 in the non-FN group, $p = 0.012$).

Patients with FN had significantly higher erythrocyte sedimentation rates (ESR) than non-FN groups on days 0, -5, and -10 (p values = 0.002, 0.048, and 0.050, respectively). Moreover, uric acid was significantly higher in patients with FN on all three days of 0, -5, and -10 (p values = 0.048, 0.012, and 0.021, respectively). Accordingly, we found a significantly lower fluid balance at days -1, -2, and -3 before BMT in the FN group (p values = 0.004, 0.012, and 0.038). Furthermore, lactate dehydrogenase (LDH) was significantly higher in patients with FN on all three days of 0, -5, and -10 (p values = 0.030, 0.006, and 0.007, respectively).

Logistic regression analysis demonstrated a significant positive relationship between the development of FN and the number of administered packed cells, the number of administered units of platelet, ESR (at day -10), uric acid, and LDH (**Table 3**). Moreover, we detected an inverse relationship between fluid balance and the development of FN. When the analysis for these variables was adjusted with baseline characteristics including age, gender, BMI, and type of lymphoma, the results of multivariate regression analysis revealed that the number of administered units of platelet, uric acid, LDH, and fluid balance were independent predictors of FN (**Table 3**). Uric acid had a positive relationship with FN, suggesting that the higher uric acid level was associated with a higher risk of FN (highest adjusted OR at day -10 = 1.617, 95% CI = 1.328 – 1.963, p value = 0.035). LDH also positively correlated with FN (highest adjusted OR at day 0 = 1.501, 95% CI = 1.198 – 2.104, p-value = 0.004). The fluid balance inversely correlated with FN, suggesting that the lower fluid balance was associated with a higher risk of FN (lowest adjusted OR at day -2 = 0.602, 95% CI = 0.299 – 0.870, p-value = 0.007). The number of administered platelet units positively correlated with FN, suggesting that the higher number of units of platelet could lead to a higher risk of FN (adjusted OR = 2.118, 95% CI = 1.010 – 2.937, p value = 0.031).

FN with profound neutropenia was detected in 41 patients (27.8%). A significant positive relationship was identified between the occurrence of FN with profound neutropenia and various factors, including the administration of packed cells, platelet units, haemoglobin (Hb), uric acid levels, and LDH (**Table 4**). An inverse correlation was also observed between fluid balance and the development of FN with profound neutropenia. After adjusting for baseline characteristics such as age, gender, BMI, and lymphoma type, the results of the multivariate regression analysis demonstrated similarly that the number of administered platelet units, uric acid levels, LDH, and fluid balance independently served as predictors for the occurrence of FN with profound neutropenia (**Table 4**).

Discussion

There is a growing concern about the widespread emergence of multidrug-resistant microorganisms in medical centres, especially in haematology-oncology wards [19,20]. These microorganisms diminish the effectiveness of routine antibacterial prophylaxis with oral fluoroquinolones in patients undergoing BMT or AHSCT [19,21,22]. In one study, discontinuing the administration of antibacterial prophylaxis in AHSCT recipients did not significantly affect the early mortality of the patients after transplantation [23]. Therefore, bacterial prophylaxis should be considered for only high-risk patients [14]. However, a risk stratification system is yet to be established for such cases. One of the reasons can be that the related and predictive factors still need to be fully recognized. Considering the close association of FN with infection in lymphoma patients receiving BMT [11], we evaluated the incidence and associated clinical and paraclinical factors of post-transplantation development of FN in this prospective study. A considerably high incidence of FN

occurred in these patients in our study, similar to previous studies' reports [24–26]. No significant differences were detected in baseline characteristics between those patients who developed FN and those who did not (non-FN); however, some other related factors were significantly different between groups, including the number of administered packed cells, the number of administered platelet units, ESR, uric acid, and LDH. We also found a significantly lower fluid balance before BMT in patients with FN. Our further analysis by controlling for baseline characteristics revealed that uric acid, LDH, fluid balance, and the number of administered units of platelet were significant predictors of FN. The prognostic value of serum uric acid for both short and long-term outcomes of admitted medical patients has been emphasized in previous studies [27]. Likewise, serum uric acid positively correlates with poor outcomes and mortality [28–30]. On the other side, a study on 112 lymphoma patients undergoing BMT revealed that LDH > 330 U/l was a significant adverse predictor of survival in these patients [31]. Reduced survival of the lymphoma patients undergoing BMT with higher LDH can be due to the higher rates of FN following BMT, which supports our findings [31]. Therefore, uric acid and LDH could be considered in future studies for risk stratification of patients undergoing BMT for the possibility of developing FN.

Another significant aspect of our findings was that higher uric acid and lower fluid balance can both suggest suboptimal hydration in FN patients before BMT. Adequate hydration is essential and can increase antimicrobial therapy results [32]. Furthermore, it can diminish the risk of urinary tract infections [32]. These findings highlight the importance of adequate hydration of the patients over the days before BMT for reducing the risk of FN.

In a similar attempt to identify the related factors of infection in patients receiving AHSCT, C-reactive protein and ferritin were proposed as predictive factors [14]. However, this study evaluated these factors only on the day of transplantation. Nevertheless, it can be more helpful if these factors are evaluated a few days before transplantation so that the clinicians have more time to modify the treatment strategies, such as bacterial prophylaxis. Furthermore, we observed some dissimilarity in the results of laboratory tests on different days (-10, -5, 0), which also impacted the extent of the relationship of these tests' results with the development of FN. This finding can highlight the time-dependent feature of the laboratory tests for predicting FN.

Other studies have identified several other related factors, including the time to platelet engraftment [33], the number of stem cells infused [34], duration of neutropenia [35,36], contamination of stem cells, presence of an indwelling central venous catheter, therapy-related mucosal damage [37,38]. However, these factors are mostly related to the post-transplantation period or during the procedure of transplantation; therefore, they may not be valuable for risk stratification and modification of pre-transplantation treatment.

We want to address some limitations of our study. The strategy for administration of bacterial prophylaxis was patient-dependent in some cases (for example, ciprofloxacin was administered only during the neutropenic days), and this issue could affect our results; however, due to the established protocol of patients' treatment and ethical issues, we were not allowed to equalize the treatment in both groups.

Conclusion

The results of our study showed that the incidence of FN is significantly high in lymphoma patients who receive BMT. A higher number of administered platelet units, higher LDH, higher uric acid, and lower fluid balance before BMT are significantly associated with FN and even FN with profound neutropenia. Accordingly, controlling the fluid balance by measuring daily input and output and adequate hydration of the patients is paramount for preventing FN in patients who receive BMT. Furthermore, uric acid and LDH could be considered in future studies for risk stratification of patients undergoing BMT for the possibility of developing FN.

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Availability of data and materials: All Data and materials collected during this study are available from the corresponding author upon reasonable request.

Authors' contributions: BN and ZK Conceived the idea.. BN, ZK, MV, and RD designed the study methodology. MF, AP, and ZK conducted the study. ZK and MF analyzed the data.. RD, AP, and BN interpreted the results.. ZK, MF, AP, and MV wrote the draft manuscript..BN and RD revised and edited the final manuscript. BN, ZK,RD,MV, and MF approved the manuscript.

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ORIGINAL PAPER

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Healthy Young POLes – HYPOL database with synchronised beat-to-beat heart rate and blood pressure signals

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ABSTRACT

Data sharing in medical research entails making research data available to other researchers for review, reuse, and collaboration. This paper seeks to describe the HYPOL (Healthy Young POLes) database, which has been prepared for sharing. This database houses the clinical characteristics and beat-to-beat cardiovascular time series of 278 individuals of Polish descent, all aged between 19 and 30 years. The data were collected from healthy volunteers who participated in multiple projects at the Department of Cardiology-Intensive Therapy research laboratory, Poznan University of Medical Sciences, Poznan, Poland. The cardiovascular time series data was obtained from non-invasive continuous finger blood pressure and ECG recordings, with sessions lasting up to 45 minutes. The HYPOL database includes an xls file detailing the main clinical characteristics and text files that capture ECG-derived RR intervals, finger systolic, diastolic, and mean blood pressure values, as well as the duration of interbeat intervals.

The data is from 149 women (53.6% of the total) and 129 men. The median age of all participants studied was 24 years, their BMI was <24 kg/m^2, pulse rate and blood pressure were average. The median duration of the recordings was almost 30 minutes. In addition, we summarise selected parameters of heart rate variability (HRV) and heart rate asymmetry (HRA).

The HYPOL database is available at hypol.ump.edu.pl. The download of data is free after simple registration. Researchers and engineers can use the database to test various mathematical algorithms for HRV, HRA, blood pressure variability and asymmetry, and baroreflex function, except for selling it.

Introduction

Data is the primary driving force behind science, offering evidence to support scientific claims and hypotheses. The discovery of new phenomena, development of innovative technologies, testing of theories, and generation of knowledge would only be possible with data. Data can be sourced through experiments, observations, surveys, or simulations. It can be analysed through diverse methods such as statistics, machine learning, or visualisation. When shared, data can be repurposed and reused [1–4].

Data sharing in medical research involves making research data available to other researchers for reuse, review and collaboration. It has received increasing attention and support from many international and national agencies, including the Organisation for Economic Co-operation and Development, the European Commission, the National Institutes of Health (NIH) and the G8 Science Ministers [1,4].

The basic idea of data sharing is that publicly funded research data is a public good that should be openly accessible with minimal restrictions, in line with transparency, reproducibility, efficiency and collaboration [1–4]. Sharing clinical and physiological research data is strongly encouraged, as it is essential for evidence-based medicine and public health policy development. Scientific, economic and ethical reasons support data sharing. Reusing and sharing data from clinical trials has also earned its recommendations and principles.

Scientifically, data sharing allows comparisons, meta-analyses and hypothesis testing, thereby increasing the validity of the data and allowing replication, which is necessary to detect falsifications and errors in the findings of other authors. It can reduce the need for redundant studies. Research funders and governmental agencies support the optimisation of resource use through the economical reuse of data. Ethically, data sharing respects the contributions of trial participants and is consistent with the idea that access to data to improve health is a fundamental right [1–4].

Data reuse saves time, accelerates progress, intensifies medical research and improves local, national and international collaboration. Data sharing also promotes scientific openness, transparency and efficiency.

Many sources provide free access to a range of clinical and physiological data. Some provide data from intensive care units, such as MIMIC-III and the eICU Collaborative Research Database, with detailed information on critically ill patients [5,6]. Other sources include cohort studies of cardiovascular health and disease, such as the Sleep Heart Health Study and the Cardiovascular Health Study with electrocardiograms (ECGs), the BIDMC Congestive Heart Failure Database, or the Apnea ECG Database [7–9].

A classic example of a multi-signal database is PhysioNet. It is a web-based resource established in 1999 under the auspices of the National Institutes of Health. PhysioNet supports and promotes complex physiological and clinical data research by providing high-quality datasets, software tools and educational materials for researchers, teachers, academics, students and clinicians [10–15].

Many of the datasets available on Physionet are examples of cardiovascular time series. In general, time series are sequences of values that change over time. Cardiovascular time series are sequences of different measures that reflect the function of the cardiovascular system, which changes with each heartbeat [16–33]. Classic examples of such series (see **Figure 1**) include:

- the duration of each cardiac cycle, measured as the distance between two consecutive R waves of the QRS complex on the ECG, is called the RR interval [16,19];
- the inter-beat interval, measured as the distance between the two systolic peaks of the pressure or pulse oxygenation waveform, is called the IBI [20];
- systolic blood pressure (SBP) and diastolic blood pressure (DBP) reflect the maximum and minimum values of the arterial pressure waveform, respectively [18,24].

Some other examples of cardiovascular time series whose values change with each heartbeat are:

- mean blood pressure (MBP) is the height of the pulse pressure time integral, calculated as the area under the pressure waveform divided by the duration of the cardiac cycle [18,21,22,24];
- pulse pressure, which is the difference between SBP and DBP [24–28];

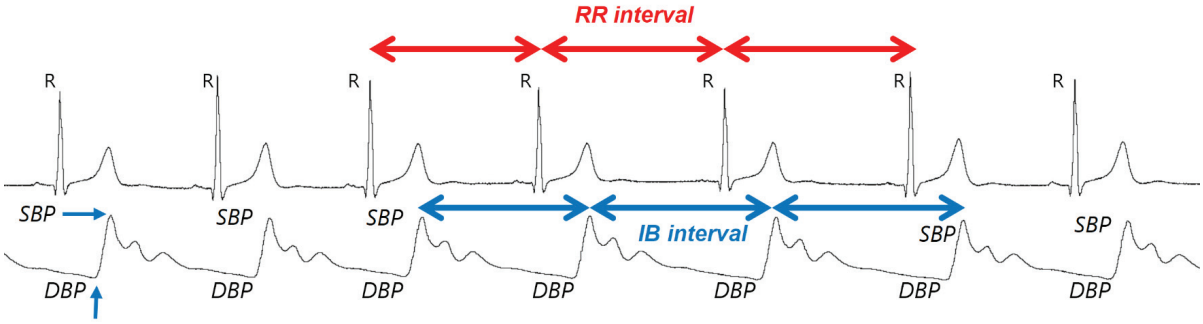

Figure 1. Example of a simultaneously recorded ECG and finger arterial pressure waveform from a healthy male. R indicates the R-waves in the QRS complex, and the distances between successive R-waves are called RR intervals. The local maxima on a pressure waveform are SBP, and the minima are DBP. The distances between the maxima of consecutive SBP waveforms are termed interbeat (IB) intervals. The pressure waveform was recorded by photoplethysmography using Portapres 2.

- pulse wave velocity, which is the rate at which pressure waves move forward in the arterial tree [29,30];
- stroke volume, which is the amount of blood ejected into the aorta by the left ventricle during a contraction [21,22];
- central venous pressure, which is the blood pressure in the venae cavae near the right atrium of the heart [31];
- vascular resistance, which is the resistance to blood flow created by the arteries and veins [21,22];
- QT interval, which is the distance between the beginning of the Q wave and the end of the T wave of the ECG [32];
- AV interval, which is the distance between the A and V peaks in the intracardiac electrograms, corresponding to atrioventricular conduction [33,34].

Cardiovascular time series can be described by mathematical parameters such as mean, standard deviation, and coefficient of variation [16, 18]. The RR intervals from the ECG are the easiest to measure and, therefore, study [16, 17]. Several mathematical methods and parameters describe the RR interval time series; this field of research is known as Heart Rate Variability (HRV). Nearly 60,000 papers have been published on heart rate variability in the last 50 years, and their abstracts are available on PubMed (**Figure 2**). In 2021, the number of published abstracts with this term was almost 4 thousand. HRV has been widely investigated in clinical, physiological, psychological, and sports studies [16,17,35–40]. Specific recommendations have been proposed for HRV [16,17].

While studying RR intervals and developing novel mathematical approaches to HRV, our

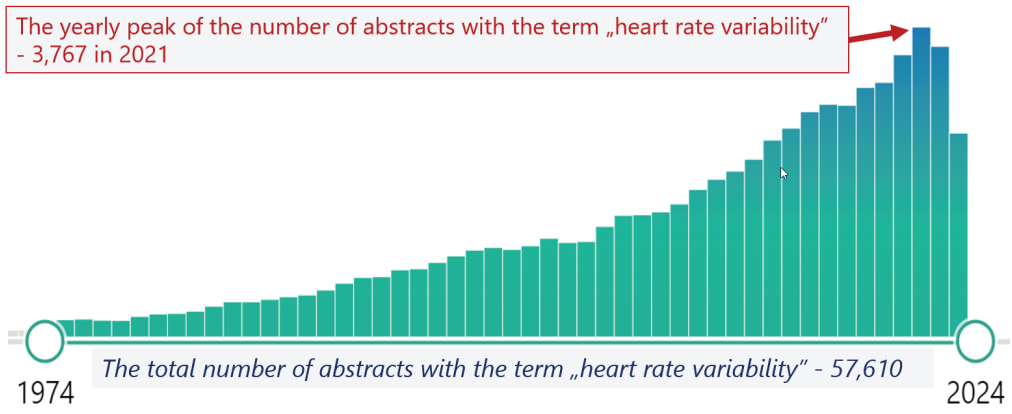

Figure 2. Trends in the number of papers with abstracts on heart rate variability in the last 50 years – data come from the PUBMED database and have been automatically generated by this service.

group discovered and described the phenomenon of Heart Rate Asymmetry (HRA). HRA is related to the unequal contributions of heart rate accelerations and decelerations to RR interval variance, structure, complexity, and trends [41–49]. We have also reported the existence of the phenomenon of HRA compensation [49]. HRA compensation is evident when heart rate accelerations contribute more to long-term HRV than heart rate decelerations do to short-term HRV.

Another example of a commonly studied cardiovascular time series is blood pressure variability (BPV) [18]. The definition of BPV is much broader than that of HRV, and repeated measures of blood pressure other than beat-to-beat are also accepted, e.g., minute-to-minute, measurement-to-measurement, or visit-to-visit [18].

Beat-to-beat blood SPB, DPB, or MBP measurements are more complicated than RR intervals from the ECG. Continuous blood pressure is monitored invasively using special catheters placed in the arteries of patients in intensive care units during some operations or cardiac wards. It is a standard procedure. However, the variability of these measurements is rarely studied. An alternative approach is to use non-invasive, usually photoplethysmography, measurements of finger SBP and diastolic blood pressure with specialised devices, e.g., Portapres, Finapres, Finometer, and TaskForce Monitor [18].

BPV has also been used in various studies and has earned its clinical recommendations [18]. In addition, we have shown that BPV also has asymmetric characteristics. SBP increases have a more significant contribution to short-term BPV than SBP decreases, which means there is another phenomenon of blood pressure asymmetry (BPA) [51].

Changes in blood pressure modify heart rate, i.e., the duration of RR intervals, via baroreceptors located in the arterial walls, mainly in the aortic arch and both bulbs of the carotid arteries [51–53]. Baroreceptors are pressure-sensitive nerve endings that detect changes in blood pressure and send reflex signals to slow or accelerate heart rate and reduce or increase vascular tone. Baroreflex function can be measured using parameters such as baroreflex sensitivity (BRS) and its delay or effectiveness (BRE) at rest during various physiological manoeuvres or pharmacological interventions [23,51–57].

Recently, we proposed a new method to study baroreflex function (ppBR) using combined Poincare plots of concordant SBP and RR intervals [56,57]. This method has shown that spontaneous short, long, and total BRS have asymmetric characteristics. Increases in SBP have stronger short-term but weaker long-term and overall effects on heart rate decelerations than on accelerations. In addition, the BRS is weaker for the interactions between SBP increases and heart rate decelerations than for the interactions between SBP decreases and heart rate accelerations. Furthermore, increases in SBP prolonged RR intervals in the short-term BPV more than decreases in SBP shortened RR intervals. In the long term, this effect is reversed: SBP decreases shortened RR intervals more than SBP increases them. The reversed effects of SBP increases vs. decreases on short- and long-term BRS demonstrated the existence of compensatory mechanisms within the baroreflex function.

Many scientists study HRV, HRA, BPV, BPA, or baroreflex function. Despite years of research, hundreds of analytical methods, and generated parameters, reference values still need to be improved. In addition, direct comparisons of different methods for HRV, HRA, BPV, BPA, or baroreflex function are rare or lacking, most likely because common reference data are not available to other scientists. In addition, some issues, such as gender differences or the effect of supine resting time, have not been fully resolved in the cardiovascular time series.

Our team has been monitoring and analysing cardiovascular time series for over 25 years, conducting dozens of studies on healthy people and heart disease patients. During this time, we have successfully collected data from thousands of individuals.

In autumn 2022, the Board of the European Study Group on Cardiovascular Oscillations and its President, Professor Alberto Porta, decided to announce a new scientific competition for 2024, "ESGCO 2024": Characterisation of sex differences in heart rate and blood pressure time series" (https://www.esgco.org/challenges). For this challenge, we have decided to share data containing different cardiovascular time series recorded at rest in healthy young men and women.

This paper aims to characterise the population studied and the data to be shared. We have,

therefore, selected a set of identically recorded ECGs and finger pressure waveforms to share with other scientists.

Study population and material

We carefully selected baseline clinical characteristics and beat-to-beat data from several cardiovascular time series from seven projects conducted in our laboratory at the Department of Cardiology-Intensive Therapy, Poznan University of Medical Sciences, Poznan, Poland. The local bioethics committee approved all projects with the following decision numbers and issue dates:

- 1144/05 of 08 September 2005,
- 1251/05 of 08 September 2005,
- 89/09 of 05 February 2009,
- 538/10 of 17 June 2010,
- 975/15 of 05 November 2015,
- 708/18 of 14 June 2018,
- 953/19 of 03 October 2019.

Most of the studies have been completed, and the last is ongoing.

All data are from healthy volunteers who agreed to participate in specific projects, gave written informed consent before participating in the study, and were informed that they could withdraw at any time. All projects were conducted following the Declaration of Helsinki [58].

Candidates were asked to abstain from alcohol for at least 24 hours and from tobacco, coffee, and energy drinks for at least 12 hours before the study. They were also asked to come to the laboratory fasting or postprandial, but at least two hours after their last meal (at the participant's choice).

The participants' health status was assessed by obtaining information on current signs and symptoms, family history, and environmental data. The history focused on chronic diseases, previous surgery and invasive procedures, acute infections, pharmacological agents and dietary supplements, drug or alcohol use and dependence, and sports training and competition participation. We excluded people with:

- known chronic illness or previous myocardial infarction, stroke, neoplasm, atrial fibrillation or flutter or after pulmonary vein electrophysiological ablation, with intracardiac occlusion, pulmonary embolism, epilepsy;
- acute infection or invasive procedure in the last three months;
- signs and symptoms of acute illness;
- chronic medication use, except oral hormonal contraception in women of reproductive age;
- pregnancy or breastfeeding in the last three months;
- drug or alcohol dependence;
- current professional endurance or resistance athletes.

Occasional use of non-steroidal anti-inflammatory drugs for pain relief (e.g., headache) was allowed, except for 48 hours before signal recording. During the routine physical examination, we always:

- took basic anthropometric measurements of body weight and height;
- obtained a standard 12-lead resting ECG;
- measured resting brachial blood pressure in the sitting position after a 10-minute rest using an oscillometric method (one of Omron's blood pressure monitors in the years 2005–2020, with two recent models Omron M5 and Omron M7 Intelli IT, Omron, Kyoto, Japan).

We excluded participants with:

- resting sinus rhythm <40 or at least 100 beats/minute, non-sinus rhythm, PQ <120 ms with features of preexcitation, channelopathies, left or right ventricular hypertrophy, QRS duration at least 120 ms, ST-segment depression or negative T waves in leads other than aVR, III and V1;
- SBP 140 mmHg or more and/or DBP 90 mmHg or more;
- body mass index <15 or at least 30 kg/m^2.

Non-invasive continuous finger blood pressure and ECG recording

All participants rested in the supine position during signal acquisition. An A/D converter—either Porti 5 with a sampling frequency of 1600 Hz or Porti 17 with a sampling frequency of 2048 Hz-recorded three channels of a bipolar chest lead ECG. TMSI (Oldenzaal, The Netherlands) produced both devices.

A non-invasive beat-to-beat finger arterial blood pressure signal was simultaneously recorded using a volume-clamp photoplethysmographic method and transferred to the A/D converter. The finger arterial pressure waveforms

were recorded using either the Portapres 2 or the Finapres Nova, both from FMS (Amsterdam, The Netherlands). Signals were recorded for at least 15 minutes, depending on the protocol and project. For the HYPOL database, we have selected signals up to 45 minutes.

Signal processing and export of cardiovascular time series

Preliminary automated analysis of the recordings was performed using the libRASCH/RASCHlab software from the libRASCH project (v. 0.6.1; http://www.librasch.org, Munich, Germany) [59]. If necessary, a visual examination of all beats and manual correction came next. ECG-derived RR intervals for each cardiac cycle with appropriate beat type annotation (regular = 0, ventricular = 1, supraventricular = 2, technical artefact = 3) and photoplethysmography-derived finger SBP, DBP, MBP, and interbeat intervals were retrieved from stored recordings. These values form the HYPOL database and can be used to calculate HRV, HRA, BPV, BPA, baroreflex function, and other analyses of cardiovascular time series.

HYPOL database

The database will be called HYPOL because it represents Healthy Young POLes, i.e., individuals of Polish ethnicity, representing the wider European ethnic group in the narrow age range between 19 and 30 years. No participant names or other sensitive data are provided; all individuals remain anonymous. The database consists of two files:

1. HYPOL DATABASE.zip, containing 278 tab-separated text files with the extension.rea. Each.rea file is from a different individual in the HYPOL population. These files contain seven columns with the following labels and data:
   - time[min] – time track expressed in minutes and synchronised beat-to-beat values of:
   - rri[ms] – duration of RR intervals in milliseconds;
   - rr-flags[] – annotation about the beat type with codes 0 for the beat of sinus origin, 1 for ventricular depolarisation, 2 for supraventricular depolarisation, and 3 for technical artefact;
   - rr-systolic[mmHg] – finger pressure SBP in mmHg;
   - rr-diastolic[mmHg] – finger pressure DBP in mmHg;
   - rr-mean[mmHg] – finger pressure MBP in mmHg;
   - ibi[ms] – duration of inter-beat interval in ms;

   Table 1 displays an example of the initial rows of the file.
2. HYPOL clinical characteristics.xls is a single Excel file with baseline clinical characteristics and file names of each individual. The columns in this file are as follows:
   - file name – the name of each record corresponding to the file names in the zipped database;
   - sex [nominal codes: "1" woman; "2" man] – with codes 1 for a woman; 2 for a man;
   - age [years] – age in years;

Table 1. A sample of 12 first beat-to-beat values from a file ag19.rea from a 26-year-old healthy woman from the HYPOL database.

time[min]	rri[ms]	rr-flags[]	rr-systolic[mmHg]	rr-diastolic[mmHg]	rr-mean[mmHg]	ibi[ms]
0.0143333333333	860.0	0	89.315797	47.274514	61.53427	899.375
0.0285416666667	852.5	0	86.846042	47.295679	62.534981	838.125
0.04321875	880.625	0	91.539302	52.472703	63.188214	1006.25
0.0601145833333	1013.75	0	91.539732	47.603983	59.600983	1186.875
0.0789479166667	1130.0	0	85.107737	43.821491	58.331134	960.0
0.0958645833333	1015.0	0	88.080992	45.398066	57.477056	1071.875
0.113645833333	1066.875	0	84.611382	41.475861	53.912648	1073.75
0.13121875	1054.375	0	79.921412	38.595414	53.739337	883.75
0.14628125	903.75	0	84.617817	41.585544	55.961219	925.625
0.16171875	926.25	0	85.355127	41.490876	64.19002	904.375
0.17728125	933.75	0	86.473242	42.82278	55.28799	1001.25
0.191322916667	842.5	0	87.400954	43.441398	54.951375	1039.0625

- BMI [kg/m^2] – body mass index in kg/m^2;
- body Height [cm] – body height in centimetres;
- body WEIGHT [kg] – body weight in kilograms;
- pulse rate [bpm] – pulse rate in beats/minute measured at the peripheral artery at the same time as brachial blood pressure;
- brachial SBP [mmHg] – brachial SBP in mmHg;
- brachial DBP [mmHg] – brachial DBP in mmHg;
- brachial MBP [mmHg] – brachial MBP in mmHg;
- brachial PP [mmHg] – brachial pulse pressure in mmHg;
- FPP – fractional pulse pressure, i.e. the ratio of PP to MBP.

Preliminary data analysis

To demonstrate the capabilities of the database, we will compute selected parameters of HRV and HRA for all participants. HRV and HRA parameters were computed using publicly available, open-source software (HRAexplorer.com). For the computation, we applied the following filters:

› only for RR intervals of sinus (normal) origin;
› the minimal accepted RR interval was 500 ms, which corresponds to the 120 beats/minute momentary heart rate of;
› the maximal accepted RR interval was 1500 ms, corresponding to the 40 beats/minute momentary heart rate.

Details on the computation and definitions of HRV and HRA parameters can be found elsewhere [15–17,23,41–43,47–49,61–67].

For HRV, we will present the following parameters [15–17,23,47,48,61–66]:

› mean RR – mean duration of all RR intervals;
› SD1 – the square root of the short-term RR intervals variance;
› SD2 – the square root of the long-term RR intervals variance;
› SD2/SD1 – the ratio of SD1 to SD2;
› SDNN – the square root of the total RR intervals variance;
› pNN50 – the percentage of successive RR intervals that differ by more than 50 ms;
› CS – the contribution of the short-term variance to the total HRV;
› CV – the coefficient of variation of RR intervals, i.e., the SDNN to Mean RR ratio.

For the spectral analysis, we applied the method of Lomb-Scargle periodograms. We used the ranges suggested by the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology [16,62–66]:

› TP – the total power in the whole frequency range (0.00–0.4 Hz) of all RR intervals;
› VLF – the power of very low frequency (0.00–0.04 Hz) of RR intervals;
› LF – the power of low frequency (0.04–0.15 Hz) of RR intervals;
› HF – the power of high frequency (0.15–0.4 Hz) of RR intervals;
› LF/HF – the ratio of the powers of LF to HF.

For HRA, we will show the following parameters [41–43,47,49,67]:

› SD1a – the square root of the short-term RR intervals variance derived from accelerations;
› SD1d – the square root of the short-term RR intervals variance derived from decelerations;
› SD2a – the square root of the long-term RR intervals variance derived from accelerations;
› SD2d – the square root of the long-term RR intervals variance derived from decelerations;
› SDNNa – the square root of the total RR intervals variance derived from accelerations;
› SDNNd – the square root of the total RR intervals variance derived from decelerations;
› C1d – the contribution of HR decelerations to the short-term HRV;
› C2d – the contribution of HR decelerations in long-term HRV;
› CTd – the contribution of HR decelerations in total HRV;
› Nd, also known as the Porta index – the contribution of the number of HR decelerations to the total number of changing heartbeats, i.e. heart rate accelerations and decelerations [47,48,67];
› CLa – the contribution of the long-term variance to the total HRV derived from HR accelerations;
› CLd – the contribution of the long-term variance to the total HRV derived from HR decelerations;
› CSa – the contribution of the short-term variance to the total HRV derived from HR accelerations;

Table 2. Summary of continuous clinical data and selected HRV and HRA parameters from the HYPOL database. Exclusively, RR intervals of sinus origin ranging from 500 to 1500 ms were used for HRV and HRA analyses.

Analysed variables	Mean	SD	Median	25th percentile	75th percentile
Age (years)	23.75	2.58	24.00	22.00	25.00
Body height (cm)	173.35	9.04	172.00	167.00	180.00
Body weight (kg)	66.49	12.33	65.00	57.00	75.00
BMI (kg/m^2)	21.98	2.66	21.79	19.99	23.80
Pulse rate (bpm)	66.67	9.23	66.00	61.00	72.00
Brachial SBP (mmHg)	111.55	10.29	112.00	104.00	118.00
Brachial DBP (mmHg)	66.64	7.12	67.00	62.00	71.00
Brachial MBP (mmHg)	80.41	7.44	80.00	75.00	86.00
Brachial PP [mmHg]	44.94	8.48	44.00	38.00	51.00
Fractional pulse pressure	0.56	0.11	0.56	0.47	0.64
Duration of the recording (min)	29.29	3.19	29.99	29.99	30.00
Sinus origin of RR intervals [%]*	96.97	1.43	96.86	96.15	97.21
Mean RR interval (ms)	893.23	113.51	893.73	807.46	961.13
SDNN (ms)	72.39	28.34	64.77	53.21	85.06
SD1 (ms)	42.54	25.82	34.94	26.12	52.67
SD2 (ms)	92.42	32.70	85.21	69.82	108.73
SD2/SD1	2.46	0.72	2.37	1.94	2.81
CS	0.17	0.08	0.15	0.11	0.21
CV	0.08	0.03	0.07	0.06	0.09
pNN50 (%)	28.88	18.76	25.63	14.16	43.71
SDNNd (ms)	49.79	18.75	45.31	37.10	58.36
SDNNa (ms)	52.51	21.35	46.36	38.26	63.58
SD1d (ms)	31.89	19.81	26.01	18.47	39.71
SD1a (ms)	28.04	16.74	23.50	18.24	34.49
SD2d (ms)	62.04	20.05	58.46	48.27	71.72
SD2a (ms)	68.35	26.23	61.56	50.55	82.72
Nd	0.49	0.03	0.49	0.47	0.51
C1d	0.55	0.06	0.55	0.51	0.58
C2d	0.46	0.05	0.47	0.44	0.49
CTd	0.48	0.03	0.48	0.46	0.50
CSd	0.09	0.05	0.08	0.06	0.12
CSa	0.07	0.04	0.07	0.05	0.09
CLd	0.39	0.06	0.39	0.35	0.43
CLa	0.45	0.04	0.45	0.42	0.48
TP [ms^2]	6322.80	6049.42	4405.63	2876.26	7484.11
VLF [ms^2]	2597.28	2387.04	1952.62	1219.79	3078.41
LF [ms^2]	1807.45	1768.45	1202.62	759.69	2201.29
HF [ms^2]	1918.08	2622.67	1035.27	573.77	2190.65
LF/HF	1.35	0.82	1.17	0.80	1.73

* The percentage of RR intervals originating from the sinus node and with a duration between 500 and 1500 ms.
Abbreviations: BMI – body mass index; C1d – the contribution of HR decelerations to the short-term HRV; C2d – the contribution of HR decelerations in long-term HRV; CLa – the contribution of the long-term variance to the total HRV derived from HR accelerations; CLd – the contribution of the long-term variance to the total HRV derived from HR decelerations; CS – the contribution of the short-term variance to the total HRV; CSa – the contribution of the short-term variance to the total HRV derived from HR accelerations; CSd – the contribution of the short-term variance to the total HRV derived from HR decelerations; CTd – the contribution of HR decelerations in total HRV; CV – the coefficient of variation of RR intervals; HF – the power of the high frequency of RR intervals; LF – the power of the low frequency of RR intervals; LF/HF – the ratio of the powers of LF to HF; Nd – the contribution of HR decelerations in number of all changing RR intervals; pNN50 – percentage of successive RR intervals that differ by more than 50 ms; PP – pulse pressure; SD1 – the square root of the short-term RR intervals variance; SD1a – the square root of the short-term RR intervals variance derived from accelerations; SD1d – the square root of the short-term RR intervals variance derived from decelerations; SD2 – the square root of the long-term RR intervals variance; SD2/SD1 – the ratio of SD1 to SD2; SD2a – the square root of the long-term RR intervals variance derived from accelerations; SD2d – the square root of the long-term RR intervals variance derived from decelerations; SDNN – the square root of the total RR intervals variance; SDNNa – the square root of the total RR intervals variance derived from accelerations; SDNNd – the square root of the total RR intervals variance derived from decelerations; TP – the total power in the whole frequency range of RR intervals; VLF – the power of very low frequency of RR intervals.

- CSd – the contribution of the short-term variance to the total HRV derived from HR decelerations;
- HRA1 – the presence of short-term HRA that is present if C1d >0.5;
- HRA2 – the presence of long-term HRA that is present if C2d <0.5;
- HRAT – the presence of total HRA that is present if C1d >0.5;
- HRAN – the presence of HRA based on the analysis of the number of heart rate decelerations and accelerations, which is present if Nd <0.5;
- HRA compensation – the simultaneous presence of HRA1 and HRA2, i.e. if C1d >0.5 is accompanied by C2d <0.5.

Statistical analysis

Graphical analysis of data distribution (histograms and Q-Q plots) and the D'Agostino-Pearson test showed that some data had normal distribution while others did not [68]. For this reason, all continuous data will be presented as Mean, standard deviation (SD), median, 25th percentile and 75th percentile. Binomial tests were used to study the presence of specific asymmetric features. Statistical analyses were performed using PQStat Software (PQStat v.1.8.4.138, PQStat Software, Poznan, Poland).

Results

There were 149 women (53.6% of all) and 129 (46.4%) men; no significant difference in sex distribution was found (p = 0.2303). The median age of all studied participants was 24 years; their BMI was <24 kg/m^2. Pulse rate and brachial SBP and DBP were regular. The duration of the recordings was nearly 30 minutes, and the mean quality showed that over 96% of all RR intervals were of sinus origin.

Table 2 summarises continuous data for clinical characteristics and measured HRV and HRA parameters.

Table 3 provides a list of the rates for different types of HRA. All forms of HRA and the HRA compensation were present in most analysed recordings.

Table 3. The rate of various forms of HRA in the HYPOL group.

HRA form	N	%	P value
HRA1	227	81.66%	<0.0001
HRA2	223	80.22%	<0.0001
HRAT	220	79.14%	<0.0001
HRAN	184	66.19%	<0.0001
HRA compensation	208	74.84%	<0.0001

Abbreviations: HRA – heart rate asymmetry; HRA1 – the presence of short-term HRA; HRA2 – the presence of long-term HRA; HRAN – the presence of HRA based on the analysis of the number of heart rate decelerations and accelerations; HRAT – the presence of total HRA.

Discussion

We provide the HYPOL database with data from healthy young adult Poles aged between 18 and 30 years who were enrolled in several previous studies conducted in our department. HYPOL database contains essential information on clinical characteristics and hundreds of files containing cardiovascular time series values, namely RR intervals from ECG and SBP, DBP, MBP and interbeat intervals measured from finger pressure waveforms. We also provide a summary of selected parameters describing HRV and HRA. These parameters have been calculated using specific filters, i.e. only RR intervals of sinus origin between 500 and 1500 ms.

As mentioned earlier, data sharing is a powerful tool that can be used to accelerate progress in medical research. By making data more available, accessible and reusable, data sharing can help researchers compare data or findings, explore problems and make discoveries. The HYPOL database allows such comparisons to be made with other databases.

The cardiovascular time series files we provide also contain different information about the duration of cardiac cycles. Although the RR intervals from the ECG and the interbeat intervals from photoplethysmography are assumed to represent the duration of the cardiac cycle, each characterises different features of cardiovascular system activity. In comparison, RR intervals reflect the distances between two peaks of ventricular electrical depolarisation, while IB intervals are the distances between two peaks of pressure waveforms. These waveforms are recorded using dis-

tinct signals, methods and sampling frequencies. For ECG, the sampling frequency in our database is 1600 Hz or 2048 Hz, depending on the version of the A/D converter. For pressure waveforms, these frequencies are 100 Hz or 200 Hz when recorded by Portapres 2 or Finapres Nova. In addition, the peaks of the R waves in the ECG are sharp. In contrast, the peaks of the arterial pressure waveforms are broader and not as well defined. The HYPOL database allows comparing RR and IB intervals in the same individuals.

Figure 3 shows the correlation between RR intervals and IBI, and **Figure 4** shows RR and IB intervals changing over time and the differences in their duration (RRI – IBI). These differences are clearly visible and not interchangeable. Nevertheless, interbeat intervals can provide valuable clinical information. As they are more readily available from sports watches, smart watches or other mobile devices, their usefulness in various clinical scenarios is being investigated. One example is the detection of atrial fibrillation [69,70].

This database has several limitations. Firstly, the data come from people of Polish ethnicity, so it is better to refer to this database in comparative studies, and any findings should be limited to a European ethnic group. Second, because of the narrow age range between 18 and 30 years, no findings should be extrapolated to children under 18 or adults over 30. Thirdly, these recordings were made under laboratory conditions in people in the supine position. The real-life recordings made outside the laboratory environment, in conditions other than those described, or in people using different pharmacological agents may differ. Fourth, the sampling frequencies of the ECGs were either 1600 Hz or 2048 Hz, which are typical for laboratories but not for other systems. Some other laboratories record ECGs at even higher sampling frequencies than we do.

The HYPOL database is primarily intended for use in the ESGCO 2024 Challenge. However, other scientists can also use the database for their studies. Use of the database is free of charge, and we would like to be acknowledged as the team that created and shared the database. A reference to the current paper would be appropriate.

The data will be available on the Poznan University of Medical Sciences website, hypol.ump.edu.pl, after potential users provide their details.

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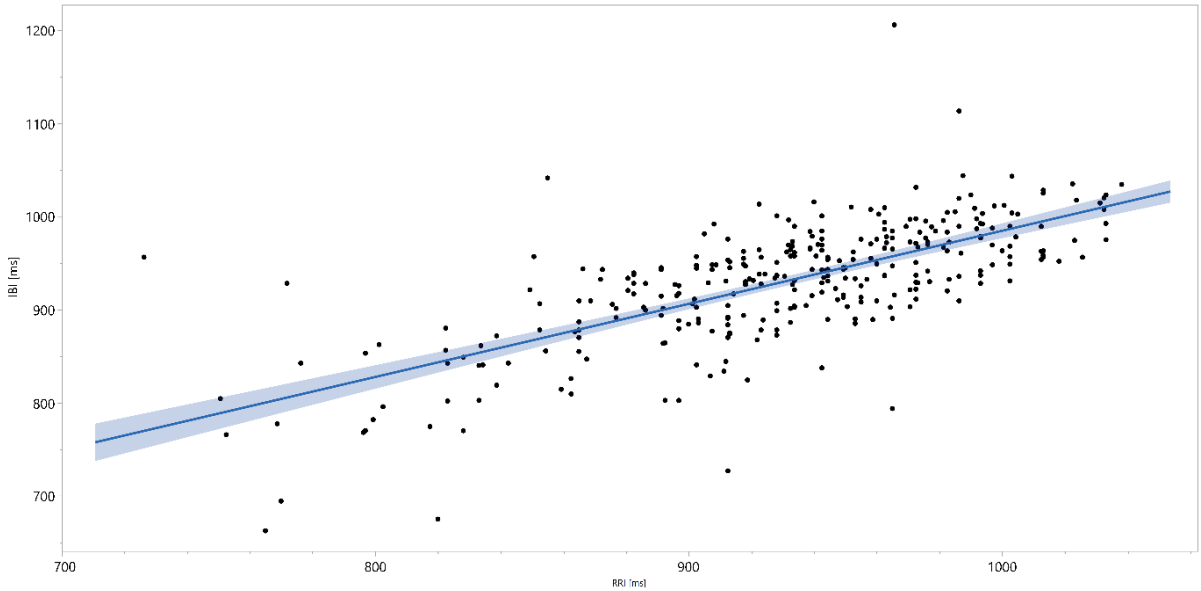

Figure 3. A simple regression line over RR intervals (RRI) from ECG and paired interbeat intervals (IBI) from finger arterial pressure waveform from the same person for the first 5 of the 30-minute recording. The value of R^2 for this regression line is 0.49 ($p < 0.0001$).

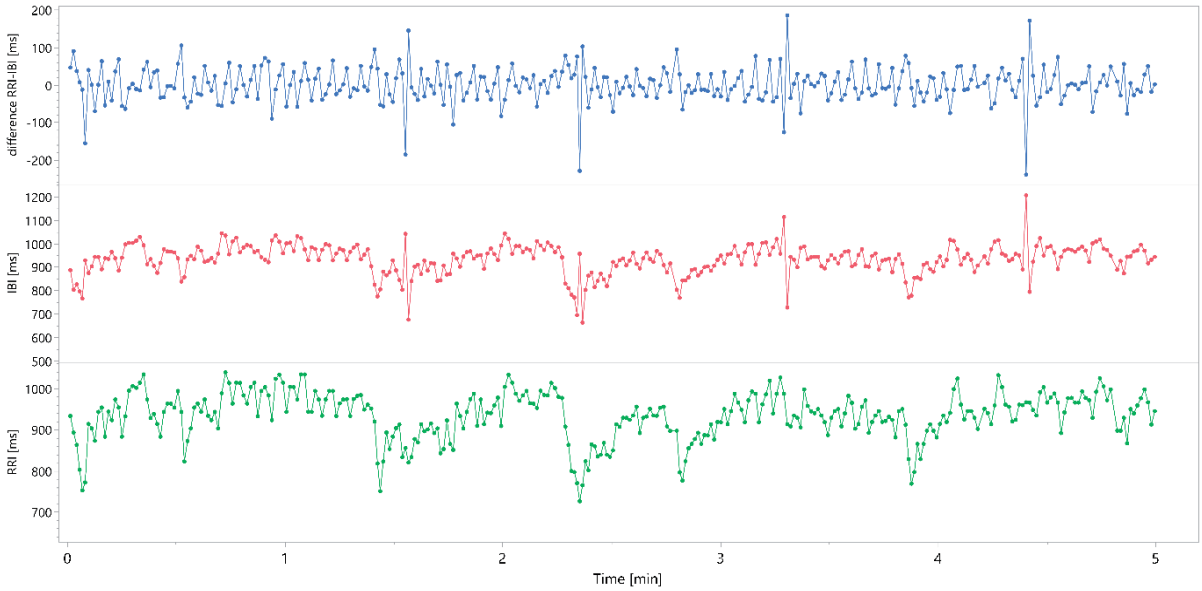

Figure 4. Local tachograms from the same healthy subject as in **Figure 3**. The lower tachogram shows all RR intervals (RRI) for each cardiac cycle recorded over 5 minutes. The middle tachogram displays the interbeat intervals (IBI) for identical heartbeats. From time to time, there are visible rapid changes in the duration of the IBIs; after a single beat prolongation, there is a sudden decrease in the duration of the IBI. A similar phenomenon does not occur in ECG and RR intervals. However, these sudden changes in the IBI are transferred to the differences in the duration of the RR and interbeat intervals, as shown in the upper tachogram. The HRV analysis results for the 30-minute recordings showed that the mean RR and IB intervals were almost identical (914.51 vs. 914.01 ms). However, other parameters differed, some substantially (SDNN – 59.12 vs. 69.37 ms; SD1 – 25.85 vs. 52.32 ms; SD2 – 79.51 vs. 82.98 ms; pNN50 – 15.94 vs. 22.67%).

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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

Study designs in medical research and their key characteristics

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ABSTRACT

Medical research study designs are many and varied. At first glance, they may be difficult to distinguish. Knowledge of their specific strengths and limitations is helpful for investigators planning new projects and for readers of the medical literature. The aims of the review are threefold: (i) to present an overview of medical research types, (ii) to attract attention to multiple characteristics of medical study designs, and (iii) to provide a concise educational resource for young researchers in the health sciences. Analysing the characteristics of medical study designs leads to achieving the goals.

Introduction

Designing a medical research project involves choosing methods to address a research question. The selection of the proper study design is critical for success and determines the limits for drawing reliable conclusions. Various types of studies have individual properties and can answer different types of questions [1]. This text briefly summarises the most common medical study designs. It also hopes to underscore several less known research design characteristics within the traditional division of study types. We refer the reader to recent reviews for novel proposals on how medical research design can be transformed or viewed from the specific perspective of personalised medicine [2,3].

Major types of research studies in biomedicine

The diversity of major study designs [4,5] highlights differences in their strong and weak points. While a cross-sectional study may be used to investigate disease prevalence, a case-control study can identify its risk factors. Furthermore, the cohort study may trace the disease course, and an interventional trial can verify if a proposed treatment works.

Of note, all of these studies are at risk of selection bias, which means that the study results can be applied (generalised) to the population of which the study group is representative. Consequently, the results of a study conducted on neonates cannot be used to conclude the treatment of older patients. Likewise, conclusions from research done only in women may not apply to males.

Observational studies

Cross-sectional study

Research of this type collects information about several characteristics of individual study participants at one time point (which does not need to be the same for each person). Then, the characteristics are summarised and compared between groups or relationships to identify potential connections. Both methods can be used, too. For example, medical students' final exam results in biophysics can be analysed in the context of their physical activity. physical activity is the exposure, and test results are the outcome. The main advantage of the cross-sectional study is an analysis of many exposures (e.g., risk factors) and outcomes (diseases, parameters). They are also cost-effective.

The major limitation is confounding, which may occur in any study. Yet, the cross-sectional design accounts for it. Confounding occurs when multiple factors coexist, and we do not know which is important. Thus, we may assume that healthy people are more physically active and often eat healthy.. However, more information is needed to say if physical activity results from health, health from activity, or both, depending on the diet. Consequently, a cross-sectional study rarely establishes cause-and-effect relationships. It is much better used to screen for correlations or to rule out strong effects.

Case-control study

The study focuses on a single outcome in this design. For example, the outcome can be the presence or absence of an inflammatory bowel disease. Patients with the disease and healthy controls can be enrolled to compare their past (usually), present, and future characteristics or either of the features.. Control participants can be matched to cases for age and other factors to minimise confounding. If the individuals are of the same age, then it is unlikely to affect the differences between the groups (though this issue has additional layers of complexity [6]). More controls than patients are often recruited to boost the study's statistical power. The result of the case-control study typically is an odds ratio, which is different from the more intuitive risk ratio (see next subsection). A more advanced view of the case-control study requires a recognition of the dynamic nature of the population [7]. The case-control design requires a high degree of organisation, and large-scale case-control studies frequently rely on long-established information technology systems. The case-control design also suffers from recall bias if information about the participants' past is obtained; people with and without disease may remember their past differently. Suppose adequate medical records and systems are available. In that case, this study enables a low-cost investigation of the relationship between a wide range of exposures and the study outcome, such as disease.

Cohort study

Time is the central concept in a cohort study. In a cohort study, many individuals are monitored over an extended period to observe the development of specific health effects, such as asthma, in a large group of children followed up for 20 years. Data about exposures and outcomes are often collected at different intervals throughout the study. A project may extend to the long-term storage of biological samples. The cohort study establishes a link between a specific exposure (such as detergent use) and later outcome (such as asthma), usually expressed as a relative risk (or risk ratio). However, such findings also need more proof of cause and effect: the cohort study is subject to confounding, even if it may be easier to mitigate than in other study types. As one cohort study may analyse multiple exposures and outcomes, extensive cohorts have been established and thus provided a wealth of data on risk factors for lifestyle diseases. Cohort studies are often large (thousands of participants). These studies offer the highest quality of evidence when the prospective design is used: starting observation of the group after all the questionnaires are ready ensures that the data are complete. Information from existing medical records or databases, i.e.,

a retrospective study, bears the risk of missing data. However, the retrospective cohort design is sometimes applied when patients with a rare disease (such as cystic fibrosis) are followed up for many years in the same centre. **Figure 1** summarises the typical timing for major types of observational studies.

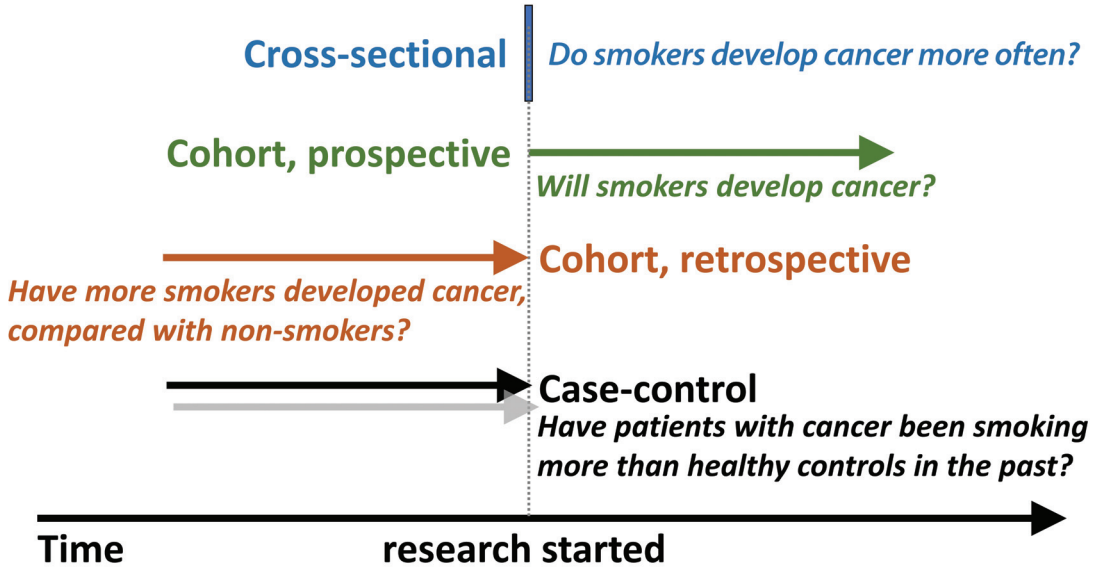

Figure 1. The relationship between major types of observational studies and time determines the types of questions that they can address. The vertical dotted line indicates the time the research was carried out. Many modifications are possible, including prospective case-control studies.

Studies of diagnostic accuracy

Assessment of a medical test's diagnostic value is a frequent research subject, often categorised separately from medical management (observational). Evaluating diagnostic accuracy requires an established test of reference, the golden standard. Comparing and assessing new methods can be done by comparing them with the reference method. Studies of diagnostic value are also subject to bias resulting from the selection of the investigated group of patients because the diagnostic value does not need to be identical in people with two different diseases. An example of a diagnostic value study is assessing of a new, non-invasive test for diagnosing early-stage lung cancer. The latest trend in diagnostic value research is embedding such studies within large interventional trials, enabling early biomarker discovery. Results from this type of research are often presented using specific metrics (such as sensitivity, specificity, positive/negative likelihood ratio, positive/negative predictive value, and the AUC – area under the receiver operating characteristic curve) [8]. Moreover, statistical inference can compare such results between various tests to establish significant differences.

Specific issues related to studies of diagnostic value

All the above-mentioned metrics of diagnostic value are useful but require careful interpretation. A key question is whether the studied group is representative of the population intended for the study results application. Sensitivity and specificity depend on the selected cut-off (threshold), which is essential yet seldom attracts attention. Sometimes sensitivity is more desirable (not to miss sepsis), while in other scenarios, specificity is required (evading false positive results in populational screening). Adapting the test for a scenario of use can often be done with the right threshold.

Studies of diagnostic value frequently report the AUC, which reflects whether the test has high sensitivity and specificity for the same threshold. Notably, an AUC of 0.5 means zero diagnostic value and an AUC of 1 suggests perfect dis-

crimination between the groups. AUC 0.7 to 0.8 is considered acceptable, 0.8 to 0.9 is considered good, and AUC above 0.9 is considered excellent. Even if the AUC is high, the test may not be good, especially if almost all analyzed data are negative or positive (group imbalance), because in such a setting, even tossing a coin would give a high AUC. AUC has also been used to summarise the performance of prognostic regression models or, more recently, artificial intelligence-derived diagnostic classifiers, but they are subject to the same limitations. Awareness of these pitfalls helps to interpret the results of diagnostic value research. Moreover, searching for other studies that show the same effects (independent replication) is useful.

Interventional studies

Randomised trial

Randomization is core to the design of interventional trials because it protects against confounding. Therefore, it enables the researcher to demonstrate cause-and-effect relationships. Random allocation of participants to a new intervention (usually medication) or neutral substance (placebo) maximizes the chances that receiving the intervention or placebo is the only difference between the two groups. Then, any observed differences can be ascribed to the intervention.

An important part of randomized study design can be using a neutral substance (placebo) or fake intervention. It removes the effect of a positive attitude brought about by awareness of a medical intervention (the placebo effect). The study is blinded if the patient does not know what intervention they received. Suppose the researchers do not know which intervention patients receive either. In that case, it is double-blinded. The method protects against assigning higher patient scores on the treatment (that scientists could prefer to show that the intervention works).

Much attention is given to the number of patients enrolled in a randomized clinical trial, how they are assigned to each group, and how many complete the study. If too many patients withdraw from the study, it may indicate a problem not predicted from the start, such as severe adverse reactions. High exclusion rates at the start of the study suggest stringent inclusion criteria or organizational problems.

There are two main ways to approach the interpretation of results from a randomized clinical trial: intention-to-treat and per protocol. In intention-to-treat, the groups are compared based on the initial assignment. If someone was prescribed the investigated intervention but had side effects and withdrew, this person will still be included in the intention-to-treat analysis. The results of such analyses are useful for physicians and insurance agencies because they link treatment effects to the prescription of intervention. Per protocol (on-treatment) analysis compares groups of patients who received study and control interventions until the end of the study. Therefore, the groups are smaller, and the comparison ignores that many patients were excluded. This analysis is interesting as it may be more sensitive to some effects of interventions but has an increased risk of bias.

Notably, the interventional study, especially the randomized controlled trial, bears higher ethical and legal requirements than the observational study, which usually incurs high financial costs. Randomized trials are registered in appropriate databases (such as clinicaltrials.gov) before they start and thus: (i) the study cannot be manipulated, (ii) it is known the study was attempted even if results are not published, (iii) other teams do not start the same expensive study that someone else is already doing. Teference textbooks summerise the clinical trial methodology [9,10]. Organizational and regulatory aspects of clinical trials are of great importance.

Randomization

The process of randomization frequently involves the use of computer tools. Block randomization typically uses pre-generated blocks of a specific size to assign patients to an intervention. If the block size is two, there are two possible blocks (orders): placebo-intervention and intervention-placebo. The randomization list consists of subsequent blocks that determine the order of group allocation [11]. This approach has a limitation: if the block size is small and the researcher knows that one patient received a placebo, she/he will be aware that the next person will be given the investigated drug. Larger block sizes disguise the intervention allocation more efficiently. However, then the group sizes may be uneven in the end. In randomized studies, study groups might

not be perfectly equal, as this results from randomization itself (unless designed otherwise on purpose). A possibility to maximize the similarity of the compared groups in confounding factors (such as sex) by stratification also exists and can be done by constructing two separate randomization lists: if the patient is female, the following allocation (placebo or intervention) is done according to the list for women (and not from the list for men). With such a setup, the study and control groups will not differ in the proportion of women. Stratification can be done with more factors, such as age, recruitment centre and medication use. Many randomized studies are multicenter, as it is often too difficult to recruit enough patients in one city and because some patients are lost to follow-up. Of note, "Mendelian randomization" does not relate to clinical trials but is an observational method to establish cause-and-effect relationships from genetic and epidemiological data analysis.

Outcomes and statistics

The time and intervention aspect of a randomized trial makes it possible to analyze trial data in multiple ways. One comparison method involves defining outcomes that can be compared at the end, such as the size of a tumour between two groups. Another approach is to check which group experiences slower tumour growth (analyzing a delta value equal to tumour size after minus tumour size before). There are other ways to approach clinical trial data, and there are also several statistical approaches and specific outcome types, such as survival, which is a complex issue. From a statistical standpoint, it is essential to appreciate one fact related to clinical trial design and sample size calculations.

In most cases, it will be challenging to statistically prove an effect of intervention when the principal outcome is defined as a binary variable (1 or 0, Boolean), like whether someone achieved a response or not. Researchers use a statistical measure called the p-value to determine if an intervention is effective. Typically, the standard assumptions for the p-value threshold (alpha or type I error) are set at 0.05 and the power at 0.8 (also known as beta or type II error at 20%). Suppose we want a statistically significant result in a trial where the first group has a 35% success rate and the second group has a 40% success rate (a 5% absolute difference and a 14% relative difference). In that case, we must recruit almost 3000 participants.. A more significant 10% absolute difference between the groups (first group 30% vs. second group 40%) would reduce the required sample size to 700 participants, which is still a large number. It is usually easier to prove the effect at a moderate sample size by measuring a continuous variable (such as blood pressure).

Sample size calculations for clinical trials are discussed by DELTA^2 guidelines (Difference ELicitation in TriAls) [12]. Surrogate outcomes are used because the most pertinent health-related outcomes are binary and because they take very long to develop (like death or stroke). Surrogate outcomes are known to be health-related, but they are easier to measure than "hard" endpoints such as mortality. Of course, endpoint use translates to what the study means for the patient and the physician, who may explain, depending on what the trial measured: "This pill will reduce your risk of death in the next ten years by 5%" or "This pill will normalize your blood pressure, which will probably help you live longer." Apart from surrogate outcomes, composite outcomes are used, where the occurrence of any endpoints is counted identically, be it death, stroke, or hospitalization. All will agree that death is not the same as hospitalization, and the two will not occur with the same frequency in the trial. Therefore, the adequate use and interpretation of composite outcomes is a challenge [13]. They sometimes need to be used because the most critical investigated outcomes occur rarely, and it is impossible to carry out extensive and long enough trials to obtain the answer. Apart from the primary outcome, randomized trials often have multiple secondary outcomes. The selection of appropriate outcomes in various diseases is not straightforward and constitutes a strand of research that quickly gains interest [14].

Commercial aspects

Many clinical trials are non-commercial, focusing on already registered medications or non-pharmaceutical interventions, such as surgery, diet, or exercise. However, randomized trials are also crucial for regulatory approval of new pharmaceuticals or medical devices. Therefore, the industry funds many and attracts additional

scrutiny because of the involved financial interests. Essential regulatory requirements accompany such trials but do not eliminate the conflict of interest risks [15].

These risks remain difficult to appreciate for the reader, as evidenced by a recent analysis of undisclosed payments to medical scientists [16]. Moreover, researchers only sometimes understand the conflict of interest similarly [17] because the related problems are diverse and not equally perceived in all cultures. Activities of the tobacco industry provided numerous and varied examples of how researchers can be influenced to evade presenting complete information, draw attention to insignificant topics or manipulate the reader and the public opinion [18]. A scientist receiving payments from a company (or owning its stock) may present biased views. Therefore, it is wise to read the medical literature critically, not ignore "conflict of interest" sections, consider authors' affiliations, and also keep in mind that study results or conclusions are often reported in misleading ways (with a "spin") [19]. However, the last of these issues overlap with a general trend towards more usage of positive language in the research literature [20]. In brief, a critical approach is always valuable while reading any research literature, including this text.

Phases of clinical trials

Table 1 lists the four main phases (I-IV) of clinical trials. Phase 0 studies may also be carried out to assess drug bioavailability and metabolism in healthy participants. Group size depends on the phase, ranging from below 100 participants (Phase I) and 100–300 patients (Phase II) to between 300 and a few thousand (Phase III).

Early Phase I investigates how the body reacts to a new substance. Phase I study carried out in healthy volunteers, defines optimal dosage and checks for side effects. Phase II investigation is a larger trial in patients that searches for evidence of efficacy and extends the safety assessment. Phase III trials are fundamental investigations of efficacy and safety and are the primary source of safety information available to physicians. Therefore, the significant difference between Phase II and Phase III is that it is still being determined if the intervention works before Phase II. Phase III studies are also more extensive and provide more precise results than Phase II. Phase IV studies are done after medication is approved and may include evaluation of treatment effects and post-marketing safety surveillance.

Simulation and in silico studies

In-silico studies help pre-clinical research, that is, computer modelling (computer processors are made of silicon).. Tools are available to predict ligand binding dynamics and off-target effects, facilitating the development of safer pharmaceutical compounds, which are then tested biologically.

Simulations may also be helpful in later phases of clinical research. Big data from electronic health records can be used to conduct simulated randomized controlled trials [21]. Such studies do not replace clinical research, but they may be used to provide additional information about the relationships between understudied population characteristics and the efficacy or safety of interventions. The primary example is ethnicity, especially when a randomized controlled trial does not reflect the entire target population well. Simulation can attract attention to phe-

Table 1. Main phases of clinical trials. The duration of studies is between several months for Phase I and a few years for Phase III studies, which are usually done in multiple centres to recruit a sufficient patients.

Phase of clinical trial	Focus
Phase I	– Choosing optimal dose – Showing safety – Healthy participants or patients with cancer
Phase II	– Showing that intervention works – Confirming safety – Done in patients
Phase III	– Confirming and measuring the efficacy precisely – Confirming and assessing safety in detail – Done in patients
Phase IV	– Monitoring safety in real life after the intervention becomes broadly available – Done in patients usually prescribed the intervention

nomena that are otherwise overlooked and may be important for specific subgroups of patients. Such computer methods (trial emulation) are complex [22].

Non-randomized interventional studies

Predominant interventional studies in biomedicine are now mostly randomized, placebo-controlled trials. However, some other interventional designs warrant discussion. One is the before-after design, applicable in uniformly progressive illnesses and when treatment is expected to yield outstanding benefits, such as gene therapy for rare diseases. Non-randomized investigation of various interventions in a real-life setting may be helpful to establish efficacy and broaden the scope of safety assessment. Some of the non-randomized interventional studies do not include a control group. Unfortunately, these quasi-experimental designs are prone to bias, so their results should be interpreted cautiously [23].

Review studies

Narrative review

A narrative review provides knowledge summarized by experts in the field without using a systematic methodology. Reviews of this type authored by most experienced researchers are often most helpful. Such reviews may serve as works of reference, similar to textbook chapters.

Systematic review

A systematic review aspires to synthesize all the information on a given topic. The goal is achieved through the application of systematic search methodology. A query is constructed and used to search literature databases. The identified articles are assessed for relevance to research questions. Quantitative information from these publications is summarized. A report from a systematic review presents the search strategy, the keywords used, the fields searched, and the databases used. Specific inclusion and exclusion criteria increase the quality of the systematic review (and the meta-analysis – see below).

Systematic review with meta-analysis

Introducing meta-analysis to a systematic review requires specific qualitative summary methods [24,25]. Studies included in meta-analysis are assessed for various forms of bias. The data available from publications and research teams are combined to obtain one more reliable summary metric of effects.

Reviewing study quality (dealing with bias) is integral to the meta-analysis process. The risk of bias is determined using appropriate tools. For randomized controlled trials, this can be Cochrane's risk-of-bias assessment tool 2 (RoB 2, [26]), which refers to potential limitations in randomization, studied intervention, dealing with missing data, approach to outcome measurement and result presentation. Other tools which can be used to assess the quality of observational studies include checklists from the National Institutes of Health or the Scottish Intercollegiate Guidelines Network (SIGN). SIGN guidelines include the quality assessment tool for diagnostic accuracy studies (QUADAS-2), which relates to patient selection bias, blinded interpretation of tests, adequate gold standard (reference) measurement, and exclusions of cases from analysis [27]. Although designed to help with meta-analysis, these tools are also valuable when planning or starting research because they guide how to conduct studies well.

There are different statistical approaches to meta-analysis, depending on the main research question. It is possible to synthesise many studies with univariate meta-analysis and compensate for study characteristics using meta-regression, which may be especially useful when data from individual patients are available to the researcher. There are also other methods which allow for linking multiple characteristics to multiple effects and for indirect comparisons (network meta-analysis).

Clinicians are often interested in the results of a systematic review with meta-analysis as this type of article provides the highest quality of evidence. The methodology of meta-analysis itself, therefore, needs to be strict; moreover, registration of a systematic review with meta-analysis is often encouraged to prevent other teams from spending effort on the same topics. More primary data is needed in many crucial areas to draw significant conclusions in meta-analyses.

Guidelines and consensus reports

We list guidelines under reviews, even though they are a separate article type. The reason for

this is the vital role of systematic search in determining the optimal medical management. After identifying and reviewing adequate references, the experts propose a set of recommendations to guide clinical practice. Most commonly, the quality of evidence to address a specific question is labelled, and voting determines what the panel will suggest as best care. However, the strength of recommendations does not rely on the quality of evidence alone, and the direct practical knowledge of the current medical practice often influences recommendations. Many guidelines cite hundreds of works; commonly, the number of referenced articles is a few times larger than in a regular review article.

Qualitative studies

Qualitative methods involve, among others, interviews, focus groups, experiments, observation, analysis of documents, and secondary sources. Qualitative and quantitative methods can be mixed [28]: e.g., interviews may be used to identify main problems, and then a survey may characterize the issue statistically. The value of qualitative studies lies in their capacity to uncover perceptions, attitudes, and mechanisms that would remain difficult to capture by quantitative research alone. They focus more on the comprehensive experience, allowing detailed and rich insights as flexible questions permit. Qualitative research makes it easier for the researcher to go beyond her/his preconceived ideas but requires much work, even to characterize small groups.

Case reports

Case reports present valuable information for learning, which is achieved through presenting typical or atypical courses of disease or unusual findings that may fuel further discovery. Case reports help identify therapeutic interventions' adverse effects and describe new diseases or risks. However, much of the information included in case reports may not be representative because of biological variability and ethnic, economic, and cultural differences, which give rise to bias. Case reports' conclusions often cannot be easily generalized, and the reports can be easily over-interpreted. The difference between a report of a series of patients and a cross-sectional or cohort study may take time to establish.

Basic biomedical research studies

This fundamental research, often involving models and experimentation [29], is behind most breakthrough discoveries fueling medical progress. Defining basic biomedical research is not straightforward, but the most commonly cited characteristic focuses on growing knowledge of nature (disease) and its mechanisms (without a specific application in mind). A cross-sectional study can meet basic research criteria when it focuses on understanding disease pathophysiology (and not necessarily treatment). It is easier to see that studies mainly involving animal models and advanced biomedical or molecular biology techniques represent basic research. Medics may overlook these pure research studies and their value because of inaccessibility (complexity) and the need for an obvious connection to medical practice. Indeed, such work is almost always of no direct value to the physician, even if it holds much promise for helping patients.

Discussion: characteristics of medical research

This article briefly introduced the main types of scholarly output in medical research. The reasons why specific research designs are more common than others may relate to the ease of conducting research under certain circumstances and the need to address specific questions. Considering the theory itself, we may see several dimensions that characterize these studies and the understanding of which may help produce unconventional study designs. Below, we list some aspects of biomedical research that are worthwhile considering while planning or appraising medical research.

The distinction between basic, translational, clinical, epidemiological, and applied research seems more related to the choice of methods than the study design itself. Like any tool, a study design can be used for different aims. Thus, both observational and interventional studies can be employed to achieve the goals of basic or clinical research. Most commonly, qualitative methods are utilized, but quantitative and mixed methods are also sometimes applied. Studies can be done only with original data, data from other projects, and both types of data sources (primary

and secondary), as is more and more commonly seen because of the availability of big data. The aspect of time further divides studies into prospective and retrospective, depending on how samples or data were gathered. Another distinction is between studies investigating, finding, and reporting new discoveries (new ideas, phenomena), proposing original solutions or hypotheses, and validation research. A prominent characteristic of the primary vs. validation research divide is between studies with small and large group sizes. The study size, in turn, is often associated with carrying out work in more than one centre or even across countries or continents. Finally, the research questions can concern various areas: determining disease frequency, understanding its pathophysiology, biomarker or treatment discovery, patient perspectives, and economic aspects. The choice of research questions and study design is pragmatic, reflecting maximum possibilities at a given budget, given the state of knowledge. Patients are also more and more commonly involved in designing research.

Medical research study design is often not a purely linear process, wherein the research question alone would determine the aims, study design, and methods. Various factors affecting individuals, groups, and consortia constrain research. Recognizing this interconnection between the research environment and the research project translates naturally to optimizing choices about details of study design and methods. Thus, planning medical research depends on researchers' expertise, interests, employment and funding, institutional, organizational, and technological capacities, responsibility for other projects, educational or commercial activity, perception of challenges in the research field, patient values and cooperation, safety, ethical and legal issues, and life situation. When planning a study, there are many factors to consider regarding its feasibility and potential impact. The most apparent trade-offs involve balancing cost versus sample size, duration versus clinical relevance and statistical power. Additionally, there may be trade-offs between a study's administrative and financial capacities and its ability to prove a cause-and-effect relationship in a randomized trial. Overall, the choice of study design is not dictated solely by the research question but remains under the strong influence of mostly unmodifiable external factors.

Additional issues include the use of proper statistics, the adequacy of the control group, and a valuable and honest section on study limitations. All research studies have limitations, but these can be overcome with proper reporting guidelines to help peers evaluate findings accurately (**Table 2**).

While commencing any medical research and while reading reports from clinical studies, it is also crucial that the pre-test probability for main hypotheses is at least tentatively assessed. If the hypothesis is implausible, it is indispensable to remain sceptical even with statistically significant results.

Table 2. Reporting guidelines for major study designs according to Enhancing the Quality and Transparency Of Health Research (EQUATOR) Network (all the guidelines are available aat www.equator-network.org). Over 600 guidelines adapted for various specific types of research are available, including economic evaluation, Mendelian randomization, pre-clinical studies, and study protocols.

Study design	Reporting guidelines	Full title of the guideline
Observational: cross-sectional, case-control, cohort	STROBE	The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies
Diagnostic value	STARD	STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies
Randomized trial	CONSORT	CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials
Systematic review and meta-analysis	PRISMA	The PRISMA 2020 statement: An updated guideline for reporting systematic reviews
Qualitative study	SRQR	Standards for reporting qualitative research: a synthesis of recommendations
Case report	CARE	The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development

The diversity of medical research designs reflects the complexity of investigated phenomena and the diversity of settings where researchers attempt to answer research questions. A reader who understands the main characteristics of clinical study designs will more easily identify the most trustworthy information in the medical literature.

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JKN: conceptualization, investigation, writing – original draft. JW: conceptualization, investigation, supervision, writing – review and editing.

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REVIEW PAPER

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Chronic fatigue syndrome – challenge in diagnosis and management: a literature review

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ABSTRACT

Introduction. Chronic fatigue syndrome is a disease that includes a number of various symptoms, among which the most characteristic symptom is fatigue. Diagnostic criteria are not unambiguous and vary depending on the scientific society by which they were developed. The aim of this review is to discuss the phenomenon of chronic fatigue, including its diagnostic criteria, epidemiology, pathophysiology, symptoms, and pharmacological and non-pharmacological strategies.

Material and methods. 45 articles published were reviewed and placed in the PubMed and Google Scholar databases.

Results. Chronic fatigue syndrome is defined as a group of symptoms whose dominant symptom is fatigue that persists after rest for at least 6 months. The Oxford or CDC criteria are most commonly used to make the diagnosis. Statistics on prevalence are inconclusive. There are several theories of origin - infectious, immunological, neuroendocrine, bioenergetic, neurological, autonomic and genetic. Other symptoms of chronic

fatigue syndrome include sleep and memory disorders or muscle and joint pain. Current treatment focuses on symptomatic treatment, including education, diet, and physical activity, as well as pharmacotherapy for pain, sleep, and cognition.
Discussion. Diagnosis and treatment of chronic fatigue syndrome undoubtedly is a medical challenge, due to non-specific symptoms, multifactorial pathogenesis and difficult to estimate prevalence of this disease. Future scientific development should focus especially on exploring the pathomechanism of CFS, which would enable the implementation of causal treatment.

Introduction

Chronic fatigue syndrome (CFS) manifests as severe, long-term, disabling fatigue associated with other symptoms, such as sleep and concentration disorders, musculoskeletal pain, headaches, frequent sore throat and lymph node tenderness [1,2]. Fatigue persists for at least six months and is not relieved by rest. Post-exercise malaise (PEM) and restless sleep accompany CFS [3]. It is a clinical diagnosis that can be made after excluding other disease processes, taking into account the characteristics of the ailments, e.g., fatigue duration, the relationship between the severity of symptoms and physical activity or rest, and triggering factors [4].

The most commonly used diagnostic criteria for CFS include the Oxford criteria and the criteria created by the US Centers for Disease Control and Prevention (CDC) [5]. They differ mainly in the number and intensity of symptoms necessary for the diagnosis, in addition to fatigue [1 Oxford criteria emphasise mainly mental fatigue, while the CDC focuses on physical symptoms [5].

According to the CDC criteria, a diagnosis of chronic fatigue syndrome can be made when there is clinical evidence of new-onset fatigue lasting for at least six months that is not affected by rest or ongoing exertion and results in a significant deterioration in previous activity level. At least four disorders are necessary for the diagnosis: significant memory and concentration impairment, restless sleep, lymph node tenderness, joint or muscle pain, headaches, and post-exercise malaise lasting more than 24 hours. Exclusion criteria include known or suspected diseases that may cause fatigue, severe obesity, depression, psychotic disorders, anorexia nervosa, bulimia, dementia, alcohol or substance abuse [1].

According to the Oxford criteria, the diagnosis of CFS requires the presence of severe fatigue lasting ≥6 months and present for more than half of the time. It affects physical and mental state and can be accompanied by muscle pain, sleep or mood disorders. Other diagnoses that may cause chronic fatigue should be excluded, as well as schizophrenia, bipolar disorder, substance abuse, organic brain syndrome and eating disorders [1,5].

In the past, the Fukuda criteria, which consist of major and minor criteria, were also crucial in diagnosing chronic fatigue syndrome. The diagnosis of CFS was possible after meeting all major criteria and ≥4 minor criteria. Major criteria include fatigue that is present continuously or intermittently for ≥6 months, was not previously present, is not significantly relieved by rest, and is interfering with the patient's daily activities. It is also necessary to exclude other causes of fatigue. Minor criteria include impaired short-term memory and concentration, sore throat, muscle pain, headache, tender axillary lymph nodes, post-exercise fatigue, joint pain not accompanied by swelling or redness, increased drowsiness or insomnia [6].

The US National Academy of Medicine (NAM) has published the updated criteria. The diagnosis of chronic fatigue syndrome requires the presence of functional impairment in the patient for at least six months, accompanied by new-onset fatigue, malaise after physical exertion, and unrefreshing sleep. It is also necessary to have ≥1 of the following conditions: orthostatic intolerance or cognitive dysfunction [7].

The Canadian Consensus Criteria (CCC) are also used. According to them, all of the following must be met: fatigue, malaise after exercise, sleep disorders, cognitive dysfunction, muscle pain, joint pain, and headache. In addition, at least one symptom from two categories must be present: autonomic dysfunction, neuroendocrine disorders or immune disorders. Symptoms are at

least three months in children and six months in adults [8].

The DePaul Symptom Questionnaire (DSQ) includes 99 items associated with CFS symptoms, disease onset and duration, energy expenditure and patient's medical history, including psychiatric history [9]. The Institute of Medicine (IOM) proposed a new name for chronic fatigue syndrome, Systemic Exertion Intolerance Disease (SEID), and criteria for the diagnosis that became less specific. The criteria included patients with mental disorders, including serious mental illnesses. For this reason, these criteria resemble the Fakuda criteria and the Oxford criteria. The SEID criteria exclude patients with pain symptoms and immune system impairment [8].

Myalgic encephalomyelitis (ME) and chronic fatigue syndrome are often used interchangeably. In 2011, the International Consensus Criteria (ICC) were proposed to differentiate ME patients from those with CFS. To meet the ICC criteria, the patient must have post-exercise neuroimmune exhaustion and ≥3 symptoms associated with neurological disorders, ≥3 symptoms associated with impaired immune function, gastrointestinal or genitourinary system, and ≥1 symptom associated with disorders of energy production or transport [10].

Diagnosis of a patient with chronic fatigue should begin with taking the medical history and physical examination, taking into account the mental state [4]. Additional tests allow us to exclude other diagnoses. According to the CDC, a urinalysis, CRP, complete blood count, TSH, phosphorus level, and a metabolic panel are recommended. According to NICE, the level of endomysial antibodies in the IgA class for celiac disease should also be assessed. Other tests should be considered depending on the patient's history and physical examination. It is essential to assess alarm symptoms that may indicate other serious diseases. Those symptoms include chest pain, lymphadenopathy, weight loss or neurological deficits [5].

Materials and methods

This review aims to discuss chronic fatigue syndrome, taking into account its epidemiology, pathophysiology, diagnostic criteria, symptoms and strategies for pharmacological and non-pharmacological management. The following keywords were used alone or in combination: "chronic fatigue syndrome", "CFS", "mtRNA", "fatigue", "treatment", "diagnosis", "criteria", "pathophysiology", "prevalence", and "risk factors". Forty-five articles were reviewed and placed in the PubMed and Google Scholar databases. Recent publications were preferred, but older references were also analysed if they brought valuable information.

Results

Epidemiology

Unfortunately, it is challenging to estimate the true prevalence of chronic fatigue syndrome. Statistical data show high heterogeneity, which results from differences in the used criteria for diagnosis and diagnostic methods, as well as the random sample of communities and age groups taken into account in the studies [11,12]. It has also been suggested that the underestimation may be due to differences in awareness among physicians and in the selection of patients in whom CFS may be suspected [13]. In addition, fatigue is a prevalent symptom, but patients meeting all the criteria of CFS are already a relatively small group [12].

Since chronic fatigue syndrome was first described in 1934 in Los Angeles, many case definitions have been developed because the pathophysiology of this syndrome remains unclear [11,14]. In a systematic review and meta-analysis of 45 articles and studies published between 1990 and 2018 in 13 countries around the world, a total of 8 case definitions were considered. It has been shown that, depending on the criteria, the incidence of CFS/ME ranges from 0.01 to 7.62%. According to data using one of the most common definitions developed by the CDC in 1988, the average prevalence was 1.46%. It has been proven that the statistics also differ depending on the diagnostic methods. The highest frequency occurred in studies based on a questionnaire (2.03%) and the lowest in medical diagnosis (0.10%).

Interestingly, some studies obtained similar prevalence results regardless of the country where the statistical data was analysed. In the synthesized data of this meta-analysis, the aver-

age prevalence is estimated at 0.89%. Although these discrepancies indicate the need for a rigorous diagnostic procedure, it can be roughly assumed that approximately 1% of the world's population, or 17 to 24 million people, suffer from CFS, which gives a similar prevalence to, for example, rheumatoid arthritis [11].

Statistical data analysis also helps isolate risk factors for developing CFS. According to research, people between 40 and 70 years old most often struggle with it, affecting women about 1.5 to 2 times more often [11,14]. Researchers speculate that hormonal factors are involved, but others point out that testing is based on medical history, and women report their ailments more frequently [11,12]. Social risk factors such as lower income and education, stressors, limited access to health care, or lack of proper nutrition are also important [11,13].

Occupational groups identified in some studies as more likely to develop CFS include healthcare workers, shift workers, airline pilots, and war veterans. Among the risk factors, viral infections are also distinguished, as they are also one of the possible pathophysiological factors of the syndrome [12]. The relationship between CFS and psychiatric disorders is also appealing. According to systematic reviews, about half of patients with CFS also suffer from anxiety and depression, or either of these. According to the criteria to diagnose CFS, some psychiatric diagnoses, including major depression, should be excluded. However, CFS and depression may co-occur [15]. Studies also report personality disorders as a risk factor for CFS [16].

Theories of chronic fatigue syndrome development

Despite many studies and scientific reports, it is not possible to identify a specific mechanism responsible for CFS development. Various theories on the aetiology of the disease are being considered, with the multifactorial nature of the disease being the most probable.

Infection theory

An infectious disease often precedes CFS symptoms, which raises the suspicion of an infectious aetiology. Studies have shown that 11% of patients with severe Epstein Barr Virus (EBV), Ross River Virus, Parvovirus B19, Coxiella burnetii, or Giardia lamblia infection will develop CFS [8]. In addition to the pathogens listed above, causative factors may also include cytomegalovirus, SARS-CoV-1, Ebola virus, enteroviruses, Borrelia burgdorferi, Mycoplasma pneumoniae, as well as fungi of the genus Candida [17]. A trigger for the disease may also be reactivation of latent infection with Human Herpes Virus (HHV-6), as evidenced by the presence of anti-HHV-6 antibodies in the IgM class and the HHV-6 antigen in peripheral blood mononuclear cells of patients [18]. Infectious agents

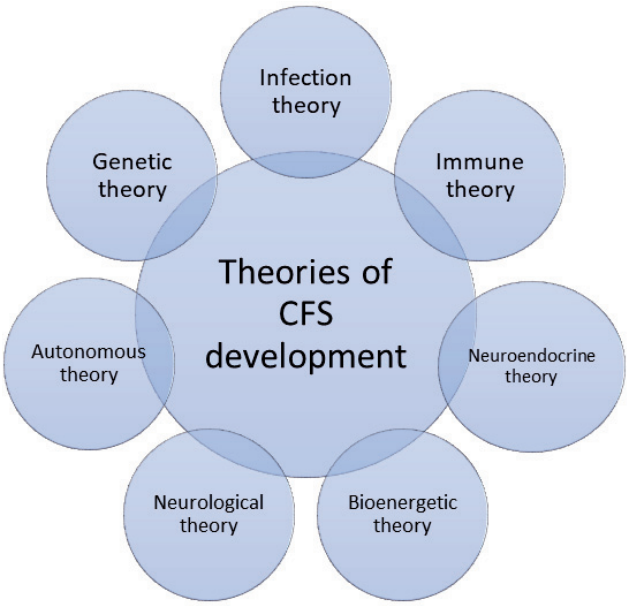

Figure 1. Theories of CFS development.

activate the nuclear transcription factor NF-kB, stimulating the immune system response [19].

Immune theory

Patients with CFS suffer from concurrent systemic inflammation and excessive immune system activation. Consequently, there is an increased concentration of pro-inflammatory cytokines such as IL-1, IL-4, IL-5, IL-6, IL-12, IL-17a, tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). High levels of pro-inflammatory cytokines are responsible for intensifying chronic fatigue, muscle and joint pain, and flu-like symptoms [17,18,19]. In the patients, disturbed function of the immune system cells, including chronic activation of CD26 T-lymphocytes, an increased number of cytotoxic CD8 T-lymphocytes, and a weakened response of T-lymphocytes to mitogens, have been demonstrated [17,19,20]. Decreased concentration and cytotoxicity of natural killer (NK) cells are also observed. In addition, their impaired functioning correlates with the severity of the disease and impaired cognitive functions in these patients [8].

In the course of CFS, humoral immunity prevails over cellular immunity [8]. Total IgG concentrations, especially IgG1 and IgG3, are reduced. On the other hand, serum levels of IgA and IgM against lipopolysaccharides of Gram-negative enterobacteria increase due to increased intestinal permeability, bacterial translocation, and serum endotoxin levels [19]. In addition, the presence of antibodies is described, mainly against nuclear and membrane structures, as well as against neurotransmitters and their receptors [18].

The pathological mechanism observed in the course of CFS is the dysfunction of one of the main antiviral pathways, which leads to the formation of an abnormal form of ribonuclease L (RNase L) with too low molecular weight. The purpose of a properly functioning RNase L is to hydrolyze the RNA of viruses present in cells. The erroneously produced form of RNase does not respond to negative feedback. As a result, it constantly destroys cell membranes, including mitochondrial membranes, which leads to damage and impairment of cell functions [8,19].

Many studies have investigated the potential use of cytokines as diagnostic biomarkers for CFS. Cytokines such as IL-1, IL-6, TNF-α, and IFN-γ have proven to be closely related to CFS. However, the level of cytokines may be different in the CNS compared to their concentration in peripheral blood vessels due to the blood-brain barrier, which is the limitation of this method. In addition, many other factors may affect the level of cytokines at a given moment, so it was concluded that they should not be used as independent diagnostic markers but only play an auxiliary role in diagnosing CFS [22].

Neuroendocrine theory

A common abnormality seen in patients with CFS, especially in women, is hypothalamic-pituitary-adrenal (HPA) axis dysfunction. The consequence is low cortisol concentration, which increases weakness and chronic fatigue [22]. Apart from low levels of adrenal hormones, in affected patients, attenuated circadian variability of cortisol and reduced HPA axis response to physical factors and stress are also observed. Adrenal hormones negatively affect the immune system and thus reduce inflammatory reactions. Similarly, reduced levels of these hormones, including cortisol, weaken the negative feedback on the immune system.

Consequently, it leads to excessive activation of the immune system and increased production of pro-inflammatory cytokines [19]. There is no clear explanation for the dysfunction of the HPA axis in patients with chronic fatigue syndrome. Chronic stress, reduced adrenocorticotropic hormone (ACTH) production, smaller size of the adrenal glands or increased negative feedback within the HPA axis may trigger the dysfunction [19,23].

Bioenergetic theory

Reduced levels of antioxidants, e.g. glutathione and α-tocopherol, increased oxidative and nitrosative stress resulting in increased levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), as well as induced nitric oxide synthase (iNOS) is observed in the course of CFS. Free radicals damage DNA, membrane fatty acids and proteins, and they cause mitochondrial dysfunction [14,17].

Damage to the mitochondria leads to impaired oxidative phosphorylation, resulting in reduced ATP production and, thus, less energy produced in the aerobic process. The phenomenon may be due to the lack of necessary substrates for this

process or mitochondria functioning impairment caused by inflammatory cells and free radicals [8]. Anaerobic metabolic pathways enable less energy production (18 times fewer ATP molecules than in aerobic conditions), cause the accumulation of lactic acid and contribute to acidosis development [7]. This mechanism favours the occurrence of the so-called PEM syndrome, i.e. malaise and exacerbation of symptoms, even after minor physical and mental effort [8].

Neurological theory

Studies of the brain of CFS patients have shown a decrease in white matter volume, possibly also grey matter, and metabolic dysfunction of glial cells. An inflammatory process characterised by widespread activation of microglia and astrocytes also occurs in the brain. These changes cause pain symptoms, impairment of cognitive functions and a decrease in the speed of information processing [7,14,17].

Autonomous theory

Autoantibodies directed against β2-adrenergic receptors have been detected in patients with CFS. Dysfunction of these receptors leads to endothelial dysfunction and excessive vasoconstriction in skeletal muscles. Muscle hypoperfusion triggers a compensatory mechanism leading to increased production of endogenous vasodilating substances that enter the systemic circulation. Consequently, patients develop hypovolemia, reduced cerebral perfusion, left ventricular preload, and decreased cardiac output. Ultimately, this leads to excessive sympathetic activation and decreased vagal tone [24]. The predominance of the sympathetic nervous system also occurs at night, disturbs physiology and makes sleep less effective [7].

Genetic theory

Genetic studies of CFS patients have shown changes in the DNA sequences and expression of many genes responsible for the immune response and the regulation of bioenergetic and metabolic pathways. In a study conducted by Billing-Ross et al. in 2016, abnormalities in the mitochondrial genome in the form of mtDNA single nucleotide polymorphisms (SNPs) were described in CFS patients. Eight SNPs located at mtDNA positions 150, 930, 1719, 3010, 5147, 16093, 16223, and 16519 have been shown to correlate with symptom severity. Increased incidence of inflammation, gastrointestinal disorders including bloating and abdominal pain, neurological symptoms such as increased sensitivity to bright light, insomnia at night and excessive sleepiness during the day were observed in patients. Patients also more often reported difficulties in performing work and limited physical activity [25,26].

Clinical presentation

CFS is also debilitating fatigue that does not subside despite rest and recovery time sufficient for a healthy person. Symptoms of chronic fatigue syndrome include malaise after exertion, non-restorative sleep, memory disturbances, muscle soreness, multi-joint pain, sore throat, lymph node tenderness, and frequent headaches. The most crucial diagnosis element is excluding organic processes [8,27].

Chronic fatigue syndrome may manifest differently in each patient. Therefore, the diagnosis is based on a group of symptoms. Fatigue is one of the most common patient complaints, especially those undergoing cancer or chronic disease treatment [1,8]. This ailment is the most frequently recognized symptom. It also noticeably disrupts daily functioning.

The pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-COV2) has increased CFS incidence [28]. Post-coronavirus disease 2019 (COVID-19) fatigue syndrome may result from damage to olfactory sensory neurons, thus leading to reduced cerebrospinal fluid (CSF) outflow through the cribriform plate and accumulation of toxins within the central nervous system.

The aetiology of CFS is different in each disease. There are no specific symptoms that occur in every patient [29,30]. CFS leads to a reduced physical, mental and social activity. The fatigue is sometimes so severe that it prevents the patient from dressing, washing themselves or climbing stairs [8].

The symptoms are so ambiguous that patients tend to associate them with a systemic disease in the first place. As a result, they seek medical advice, usually from a general practitioner [9,10]. In cases when the symptoms are intractable, patients ask for help from a psychiatrist. That is especially true when severe CFS causes percep-

tual and sleep disturbances and difficulties in understanding complex sentences. In addition, CFS symptoms may occur or be more visible due to depression, hypotension or disorders affecting sleep quality and depth. The onset of chronic fatigue syndrome symptoms is recognizable, so the patient can determine up to what point their functioning was normal [1,8,27].

The most characteristic CFS symptoms have been mentioned above. However, it should be noted that the clinical course varies. Other symptoms include allergies and food hypersensitivity, diarrhoea, bloating, dry eyes, dizziness, earache, night sweats, jaw pain and numbness or tingling in the face, hands and feet [1,31].

What is more, CFS often coexists with other autoimmune diseases, which is why the blood may contain, among others, antithyroid antibodies, rheumatoid factor or anti-smooth muscle antibodies [21]. Fatigue, depending on its duration, can be classified as acute (lasting less than one month), prolonged (between one and six months) and chronic (at least 6 months). Acute fatigue resolves with rest, while chronic fatigue may indicate idiopathic chronic fatigue or chronic fatigue syndrome. Chronic fatigue syndrome differs from chronic fatigue of other causes as it is a systemic neuroimmune disease with a different pathophysiology. Chronic fatigue of unknown causes also characterises idiopathic chronic fatigue, but the CFS criteria are not met [32].

CFS should be differentiated from other abnormalities causing fatigue. However, CFS also often coexists with other diseases like connective tissue diseases. Fibromyalgia was once considered a CFS spectrum disease. However, differences in sleep architecture patterns have been shown between patients with chronic fatigue syndrome and fibromyalgia and those with only CFS. It is essential to differentiate fatigue resulting from CFS from fatigue found in other disorders [33]. A diagnosis of CFS is a diagnosis of exclusion. It requires the presence of fatigue lasting at least six months and concomitant symptoms, such as cognitive impairment, unrefreshing sleep, body pain, and post-exertional malaise – PEM [33,34]. Exertion and other stressors exacerbate those symptoms. Malaise after minimal physical or cognitive exertion characterises PEM [34]. PEM is the most indicative of CFS and is a hallmark symptom [34, 35].

Pharmacological and non-pharmacological strategies in treatment

An effective causative CFS treatment remains unknown due to its complex and inexplicable aetiology. Treatment focuses on symptom alleviation through pharmacological and non-pharmacological methods (**Figure 2**) [7]. Patient care based on a multidisciplinary approach is required. The management strategies include patient education, symptomatic treatment, appropriately adjusted physical activity, body's energy management and in some cases cognitive-behavioral therapy (CBT) [36–38].

Education about the condition plays a significant role in the therapeutic process. Information provided to the patient and family should be understandable and tailored to the patient's situation. CFS symptoms are variabile, represent heterogenous course, and may affect different aspects of a patient's life [36,37]. Knowledge of the body energy reserves among patients with CFS is essential.The patients need to acknowledge their limitations and learn to manage energy appropriately. Energy self-control reduces the probability of PEM and exacerbation of other symptoms [36].

The clinical course and the patient's willingness to exercise determine the decision to incorporate physical activity into the treatment process. A physiotherapist should tailor and supervise the training plan with adjustments to the patient's energy levels. The patient must be aware of the risk of symptom exacerbation during activity. National Institute for Health and Clinical Excellence (NICE) guidelines currently do not recommend graded exercise therapy (GET) [36].

There is no approval for the cognitive-behavioural therapy as a CFS treatment method. It plays only a supportive role. Considering the possibility of overlapping symptoms of CFS and other diseases is necessary, as it may create a diagnostic challenge [36].

Patients with multimorbidity and comorbid CFS require particular care. Treatment of concomitant diseases should follow guidelines. The possibility of overlapping symptoms of CFS and other diseases needs to be taken into consideration, as it may create a diagnostic challenge [36,37].

Non-pharmacological strategies include proper nutrition, dysautonomia's symptoms alleviation, sleep disorder, cognitive dysfunction and pain relief therapy. A balanced diet and

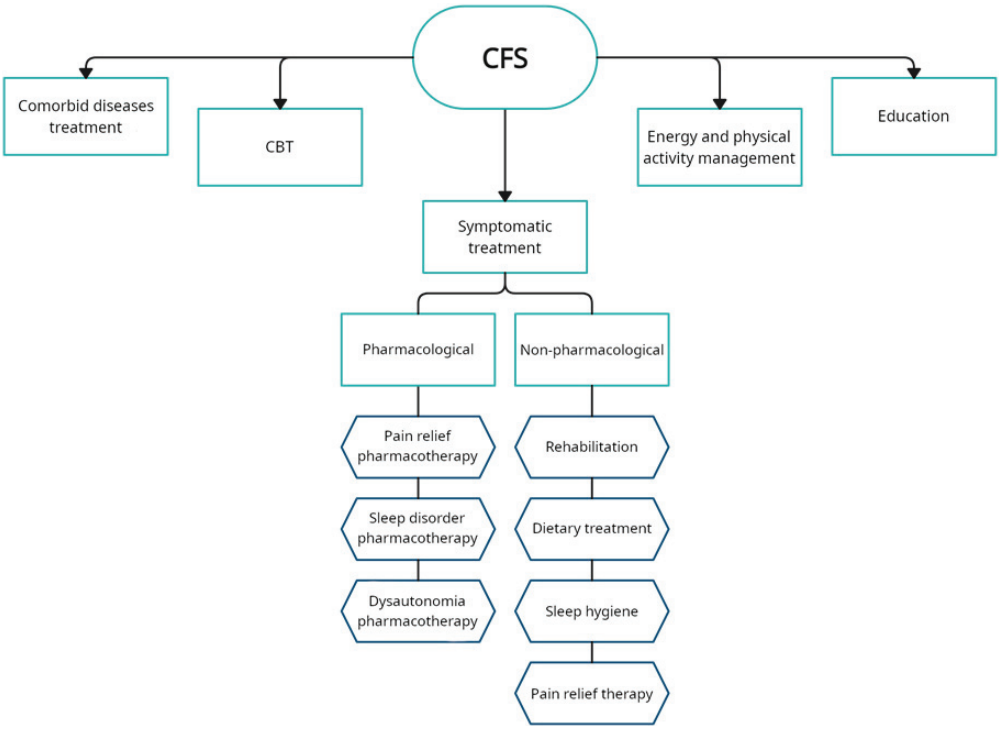

Figure 2. Treatment focused on symptom alleviation through pharmacological and non-pharmacological methods.

appropriate hydration provide the basis of nutritional treatment. Additionally, low-carbohydrate, high-protein, rich in omega-3 fatty acids and anti-inflammatory diet may be considered [37]. A dietician should supervise patients having difficulties with maintaining average body weight. At risk of vitamin D deficiency are patients whose severe CFS symptoms force them to limit activity or cause immobilization. Therefore, vitamin D supplementation is recommended in accordance with the guidelines [36].

Providing proper quality and quantity of sleep and rest between activities during the day is essential to CFS management. The patient should learn that sleep disturbances exacerbate fatigue [36,37]. Helpful tools for regulating patients' sleep include phototherapy, relaxation techniques and blue light filters [7].

Symptoms resulting from autonomic system dysfunction can be alleviated by increasing fluid and electrolyte intake, using compression stockings, sleeping with elevated legs, and avoiding prolonged verticalization [7,37].

Pain management includes physiotherapy, acupuncture, acupressure and warm and cold compresses [7,37].

Patients affected by CFS require help in daily activities. In severe cases, there is a particular risk of physical functioning deterioration due to immobilization. Methods that improve muscle flexibility, joint mobility and balance and positively affect the cardiovascular system are recommended [36].

Adjusting the intensity of mental activity to the patient's capabilities, for example, focusing on doing one activity at a time, and using memory aids such as notes or a calendar, can be helpful in improving cognitive function [7].

A conversation with the patient should precede the initiation of treatment. The patient should determine which symptoms are most burdensome and disruptive to daily functioning. The treatment plan and decision to include pharmacotherapy should be individualized and tailored to the type of current symptoms and their severity [8,39]. Since no pharmacotherapy aims directly against CFS, drugs are an auxiliary intervention to alleviate symptoms. There are no specific indications for pharmacotherapy. The outcome of pharmacological treatment may be different in each patient. One patient may benefit from drugs that relieve symptoms. Howev-

er, these may not be effective for others. When managing the symptoms, it is advisable to start pharmacotherapy with over-the-counter drugs before including prescription drugs. Therefore, medical professionals should support and supervise the patient's condition during the treatment [40,41].

Pharmacological treatment reduces pain, dysautonomia symptoms, sleep and cognitive dysfunction. Some anti-inflammatory and anti-allergic drugs may also be administered [37]. Due to the higher risk of developing drug intolerance, CFS patients should start therapy with a lower dose and increase it gradually [36]. Pain management includes paracetamol, nonsteroidal anti-inflammatory drugs, low-dose naltrexone, antiepileptic drugs, serotonin and norepinephrine reuptake inhibitors. Reducing orthostatic intolerance can be achieved by including fludrocortisone, low doses of beta-sympatholytics, alpha-receptor agonists and intravenous saline. For sleep disturbances, trazodone, antiepileptic drugs and low-dose antidepressants are recommended. Cognitive impairment in patients with CFS may be treated with methylphenidate or dextroamphetamine, but their addictive potential should be kept in mind. The literature mentions modafinil as well [7,37].

The effectiveness of vitamin and mineral supplementation in treating CFS symptoms has not been confirmed [31]. According to an analysis by Bjørklund et al., vitamin A and E deficiency may play a role in the pathophysiology of CFS. However, further research is needed to confirm this thesis [42]. The research on potential drugs is possible because of increasing knowledge of the aetiology and pathophysiology of CFS. Experimental therapies targeting immune and mitochondrial dysfunctions are being developed [43].

Promising results were obtained during a study conducted by Kujawski et al. It focused on the effect of stretching exercises combined with systemic cryotherapy. The research proved that this method could reduce the sleepiness and fatigue experienced by CFS patients. Improvements in some cognitive functions were mentioned as well [44].

According to the EUROMENE consensus, in the absence of targeted treatment for CFS, the most important thing to do is to manage by avoiding overexertion and mental stress, activities that can lead to symptoms. Physical activity should account for two-thirds of the duration and intensity that usually causes symptoms. Thus, patients with CFS should first and foremost be adequately educated in appropriate energy and physical activity management [37]. Also, in a review of national recommendations in European countries, the most commonly recommended treatment procedures are appropriate exercise management and CBT [45]. Hence, patients with CFS and without comorbidities should first be adequately educated about CFS, how to manage energy, adjust exercise and avoid mental stress, with which CBT can help. After that, pharmacological and non-pharmacological treatment of symptoms should only be considered.

Discussion

In our review, we wish to emphasize the care with which CFS should be diagnosed and treated. More than simply matching the diagnostic tools used can be problematic. Several uncharacteristic symptoms, often difficult to assess objectively, and often the need to base the diagnosis on the patient's subjective feelings, may delay the diagnosis [1,31]. One of the most essential elements of management is excluding organic processes that may cause such a condition in the patient [8]. The progression of this syndrome, characterized by severe and prolonged fatigue, can lead to disability, so it is crucial to develop, refine and implement new diagnostic methods for CFS [1].

As we wrote above, it is difficult to estimate the actual incidence of CFS due to the multiplicity and imperfection of diagnostic criteria or insufficient medical staff education [11–13]. According to our literature review, the estimated prevalence of CFS can be compared to that of rheumatoid arthritis, which, however, is a disease with a more straightforward diagnosis and awareness among patients and physicians of the symptoms of this disease is broaderr than that of CFS [11].

Despite many years of research and attempts to discover the pathomechanism of CFS, the aetiology of this syndrome remains unclear and is suspected to be of multifactorial origin. As we indicated above, there are various theories of the pathophysiology of CFS, but none thoroughly explains the occurrence of all symptoms. Further

research on this topic is needed to develop targeted therapies.

So far, CFS therapy is based on symptomatic treatment. Due to, as we mentioned, the multiplicity and uncharacteristic symptoms, this treatment requires a multidisciplinary approach [7]. Research emphasizes the importance of educating the patient about their disease and cooperatively developing appropriate management of the body's energy resources, including adapted quantity and quality of physical activity [33,34].

In the care of patients with CFS, it is also necessary to treat comorbidities that may worsen the course of CFS and to provide patients with ongoing and long-term care so that the symptoms of CFS do not mask any possible development of other conditions [33,34].

Our study shows that CFS is a complex problem with imperfect diagnosis, requiring careful research into its pathophysiology and possible causal therapies. It is also essential to raise public awareness of the syndrome and to adequately educate medical professionals.

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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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Photodynamic therapy applications – a review

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ABSTRACT

Photodynamic therapy (PDT) is a treatment method gaining worldwide attention. The paper overviews studies on PDT, as it may be applied in many medical disciplines, such as dermatology, urology, gynaecology, the therapy of head and neck cancers, and age-related macular degeneration. Recently, the development of this method has sped up, which is related to the appearance of new photosensitizers and optimised dosimetry of light. Current studies indicate that PDT is a significant support for conventional treatment protocols in many cases.

Introduction

Photodynamic therapy (PDT) is a modern medical method for both diagnostic and therapeutic purposes [1–4]. It is used to combat various external and internal diseases. In PDT, a specific wavelength of light is used to excite the photosensitizer (PS), which helps make reactive oxygen species, such as singlet oxygen. A potential PS for PDT has to fulfil numerous requirements that guarantee the safety, effectiveness, and competitiveness of the treatment. It means that potential side effects related to PS are limited. Moreover, PS should be selective towards pathologically changed cells and non-toxic towards healthy ones. PS manufacturing costs are also essential [1,5,6]. Intensive research on PS preparation and potential applications with high affinity for diseased tissues is underway. One can increase PS selectivity by combining antibodies or nanoparticles with the PS molecule [7]. PSs have also been applied in medicine in diagnostics, especially in photodynamic diagnosis – PD and therapeutic methods – PDT [8]. PDT is a medical treatment that uses light-sensitive drugs called PSs, administered locally or systemically. After irradiation

with light of an appropriate wavelength, the excited PS molecule can transfer the energy to neighbouring molecules [9–12]. Thus, the excited PS molecule can transfer the energy to oxygen molecules, forming reactive oxygen species (ROS) like singlet oxygen, hydroxyl radical, or superoxide. ROS can cause several effects in cells, e.g., damage to cellular membranes or organelles, such as mitochondria or nuclei. It could lead to the activation of apoptosis or necrosis pathways [13,14]. The most desirable are PSs with maximum absorption in the so-called therapeutic window between 600 and 850 nm [15]. An appropriate laser or LED lamp is usually recommended to induce fluorescence in PD. The light source emits a wavelength of around 400 nm (near UV range/blue light), which results in the red fluorescence of a contrasting agent (PS). The identification of malignancies or lesions is possible through the accumulation of PS molecules inside affected cells. [13]. The PD has an advantage over other optically assisted diagnostic methods because of the fluorescence that makes lesions more visible due to the selective uptake of the PS molecules. This phenomenon grants PD high specificity and sensitivity, compared to other more conventional diagnostic methods (regular cystoscopy or dermatoscopy) in white light [16]. Faster diagnosis provided by PD allows for detection in the early stage of the disease and establishes the correct treatment protocol [14].

Photodynamic methods for dermatology

One of the most severe skin diseases is cutaneous melanoma, which constitutes approximately 1.7% of all malignant cancers diagnosed annually worldwide. The survival and outcomes of the treatment are strongly tied to the development stage of the malignancy, thus making regular screenings a critical aspect of preventive actions. The risk of melanoma occurrence is higher when the patient meets the following conditions: a fair skin type, multiple atypical moles, or a family history of melanoma. The most frequently used screening method is a complete body-skin examination using dermoscopy or other imaging methods, which requires a trained physician [17]. In light of the need for a fast and reliable method of skin examination, the phenomenon of melanoma cell auto-fluorescence was considered. As the melanoma tissue contains molecules that have the character of fluorophores (i.e., lipofuscin and melanolipofuscin), they can be easily differentiated from the healthy tissue in a non-invasive manner [18].

Considering the treatment strategies, traditional surgical excision is still the most common way to deal with topical lesions, as it applies to many malignancies at various developmental stages [17]. However, this approach reveals some limitations due to its highly invasive nature and limited selectivity, resulting in a high-margin area of healthy tissue that must be removed along with the cancerous tissue. As a topical skin disease, melanoma can be treated using PDT as an alternative method to traditional surgical treatment [19]. The main advantages of PDT are the appealing cosmetic effects left after the treatment and its high selectivity for malignant cells. Some limitations of PS for PDT can be further improved with nanocarrier technology. Some limitations of PS for PDT can be further improved with nanocarrier, technology. PDT was successfully used in treating IV-stage metastases of pigmented melanoma with chlorin e6 (Ce6) as the PS, resulting in an excellent outcome, no recurrence, and no after-treatment toxicity [19]. Another advantage of Ce6 is that it can be used parallelly as the PS and as the imaging agent coupled with a nanocarrier, thus leading to a precise, real-time, two-colour image with the green fluorescence protein (GFP) expressing melanoma cells of the treated region.

More importantly, this strategy also allows for the early prediction of the treatment outcome, providing information on the deposition of the active agent [20]. What needs mentioning is a possible connection between melanin content and the classical PS molecules. As melanin *in vivo* displays strong absorption in the range of 500–600 nm wavelength, it may compete with some PSs, e.g., Photofrin, which reveals absorption around 630 nm [19]. However, replacing the PS removes the inconvenience easily. For example, bacteriochlorines can be used as they reveal the characteristic Q band long-wavelength absorbance at around 770 nm that can be even further extended with different molecule transformations by adding ligands, chelating metal ions to

the core, or further modifications of the macrocyclic system, leading even to bacteriopurpurinimides with the maximum at 836 nm [21]. In this case, they present absorption maxima that do not overlap with the absorption range of the melanin and thus do not affect the efficiency of the potential treatment.

One of the key aspects influencing the effectiveness of PDT, primarily when the therapy targets the circulatory system, is the degree of oxygenation of the tissue in which the process occurs [22]. Damage to blood vessels can rapidly disrupt the delivery of oxygen to tissues and thus significantly reduce the effectiveness of PDT. Photodynamic therapy directed against some cancers in such a situation may be self-limiting. Also, in many tumours, the degree of oxygen supply to the tissues can be very inhomogeneous due to the chaotic and pathological process of angiogenesis, leading to an unevenly distributed activity of the photosensitizer, which is directly dependent on oxygen [22]. From this perspective, developing protocols enabling PDT to operate even in unfavourable conditions remains fundamental. Over the last few years, it has been proposed to implement several modus operandi, e.g., using interval exposure and chemical oxygen sources such as hydrogen peroxide. Another approach to this issue is developing photosensitisers that will generate ROS not only by type II photodynamic reaction but also by type I (less sensitive to oxygen concentrations) and type III (practically insensitive to the presence or absence of oxygen). While the number of photosensitisers based on the type III photodynamic reaction is still limited, the development of molecules generating ROS in the I and II photodynamic reactions is auspicious [23,24]. Bacteriochlorins require special attention in this context. They have many desirable features, such as high photostability, a relatively long lifetime in the excited state, and high quantum efficiency of oxygen generation. In addition, bacteriochlorins are characterised by low dark toxicity and light activity, even at low concentrations [25]. An essential aspect, however, is their ability to generate ROS by applying type I and type II mechanisms.

Zhu et al., who compared the activity of chlorins vs. bacteriochlorins vs. porphins, presented an interesting perspective. Their study indicated that bacteriochlorins revealed the highest absorption band, were the most effective anticancer agent, and simultaneously had the lowest dark toxicity in all compared macrocycles [26]. Also, in the case of skin cancer, formulations based on bacteriochlorins have brought a significant breakthrough in PDT. For a long time, the scientific community was sceptical about the use of photodynamic therapy in the treatment of melanoma. First of all, radical resection of the neoplastic lesion with a large margin of normal tissues has been considered the therapy of choice for many years. A significant challenge was melanoma cells' high concentration of endogenous pigment compounds. Their presence significantly reduces the interaction of lower-wavelength light with the photosensitizer. Therefore, using bacteriochlorins, whose absorption maximum is usually over 700 nm, proved an interesting possibility. Mroz et al. conducted ground-breaking research in 2010 on the use of bacteriochlorins to treat melanoma. Their intervention in a mouse model provided a significant survival advantage, with 20% of cures [27]. Experiments using redaporfins in Pluronic P123 produced even better results. As a result of the protocol, obtaining even a 100% long-term cure rate in B16F10 tumour-bearing mice was possible. Considering these results, the use of bacteriochlorins in treating skin lesions has up-and-coming prospects [28].

Photodynamic methods for acne treatment

Acne vulgaris is a long-lasting, inflammatory skin condition that many things can cause, mainly hyperseborrhea (excess sebum), environmental factors, dietary choices, smoking, stress, and bacterial infection with *Cutibacterium acnes* [29]. PDT is another way to treat this condition. The application of PDT allows for reducing the number of C. acnes colonies and down-regulating sebum secretion, but the entire mechanism is unknown to date [30]. Despite that, using PDT against acne exhibits promising results, especially in severe and moderate cases resistant to conventional systemic treatment strategies with antibiotics or retinoids [30]. For this purpose, aminolevulinic acid (ALA) is mainly used and researched as the PS-protoporphyrin IX (PPIX) precursor. The idea behind the phototoxic effect after ALA is applied

to the skin is that this simple molecule is a building block for heme during its biosynthesis pathway. As the topically applied ALA enters the cells, it is converted in a few steps to the PPIX, which accumulates since the ferrochelatase (the ferrous ion-inserting enzyme – creates heme) is the slowest-acting enzyme of this biochemical pathway, thus rate-limiting. The PPIX has characteristic red fluorescence and, when sun-exposed, acts as a PS [31]. As the ALA molecule is relatively polar, its permeability must be considered mainly because of the lipophilic character of the *stratum corneum*. However, this may also be its advantage since some types of lesions caused by acne tend to exist close to the surface of the dermis, resulting in easier permeability for the targeted regions [32]. In the case study of ALA-PDT, the combination of 10% ALA cream and red LED light (630 nm, 40–80 J/cm^2) was applied against severe acne in three PDT sessions performed one week apart from each other, resulting in significant improvement after one month with a persisting effect up to four months after the last treatment [30]. The side effects of this treatment were mild exfoliation, erythema, and mild oedema lasting about 2–4 days after the procedure. What is worth mentioning is that no additional scarring or pre-existing scars changed significantly. Moreover, an improvement in skin texture was reported [30].

Photodynamic methods for the treatment of oral leukoplakia

Oral leukoplakia (OL) is a pathological lesion originating from the mucosa of the oral cavity with significant potential for developing malignancy [33]. OL is a disease that benefits from developing treatment strategies since there is no clear evidence of effective treatment preventing cancerous transformation or the recurrence of OL [34]. One of the advantages of PDT is that it only affects treated regions, making it a non-invasive way to treat premalignant lesions. A 20% Ce6 and 10% dimethyl sulfoxide gel with an occlusive dressing was put directly on the damaged mucosa and the healthy tissue around it for an hour before the light treatment to see if PS Ce6 could be used. Illumination was performed with a semiconductor laser at the wavelength of 660 nm. The procedure was repeated ten times at two-week intervals, resulting in a significant mean lesion area and reduction of lesions to 79.3%. The overall efficacy of the treatment was noted at the level of 70.9% for the non-smoking group, whereas the smoking group did not respond to the treatment [33].

Besides Ce6, ALA can be used to treat the OL. For example, a 20% ALA gel was applied to the defective mucosa 2 hours before the treatment. A 395 nm UVA flashlight was used just before the treatment to check for the presence of the PPIX in the treated regions. A lesion that had exhibited red fluorescence (giving evidence of PPIX presence) could undergo the laser treatment. Local oral anaesthetic medicine – primacaine was administered prior to irradiation with light at 632 nm to decrease discomfort during the laser therapy. As a result, a high positive response to ALA-PDT was observed in 86.2% of the patients. The rest, 13.8%, responded little [34].

Photodynamic methods for urology

Prostate cancer constitutes one of the most commonly occurring malignant tumours worldwide in men, the second after lung cancer [35]. The main symptoms of prostate cancer are discomfort and difficulties during micturition, urine incontinence, pollakiuria, dysuria, hematuria, bladder pressure, and a narrow stream of urine [36]. Compared to trans-rectal ultrasonography or digital rectal examination, finding out the levels of prostate-specific antigen (PSA) in the blood is the best way to diagnose. However, if any of these examinations reveal an abnormality, prostate biopsy or multiparametric magnetic resonance imaging is recommended to properly locate and estimate the character of the malignancy [37]. The treatment options for high-risk prostate cancer are external beam radiation therapy, long-term androgen deprivation therapy, and radical prostatectomy, the last one being especially invasive and associated with many after-treatment unwanted outcomes such as nerve or muscular tissue damage involved in excretory or erectile functions resulting in, i.e., impotence, affected micturition, incontinence [35,38].

PDT may meet these clinical needs, strongly improving selectivity and, for some PSs, allowing the performing of *in vivo* image-guided PDT,

potentially improving the accuracy and the outcome. For increased selectivity, the PS molecules (Pc413 and IR700) were conjugated with peptide targeting the prostate-specific membrane antigen (PSMA), providing high-affinity PDT agents with a binding force greater than 4.6-fold compared with related Cys-CO-Glu ligand. Comparing the imaging efficiency *in vivo*, the PSMA-1-Pc413 demonstrated a much clearer image and accumulated in the murine prostate region. However, after PDT, both conjugates demonstrated significant tumour regression [38]. Another PS used for prostate cancer was padeliporfin (Tookad®), used as a vessel-targeting agent in the type of focal therapy called vessel-targeted PDT. The technique depends on the intravenous administration of the PS, resulting in the systemic presence of the pro-drug and localized activation of the PS by exciting it with appropriate light provided by optical fibres inserted into the affected prostate regions [39,40]. This approach provides excellent outcomes for patients with low-risk prostate cancer, being effective, safe, and easy to perform in ambulatory conditions and also giving the patient the comfort of being discharged on the same day of the procedure. The limitation of this approach is related to the systemic administration of the PS, making it mandatory for the patient to avoid direct sunlight exposure up to 48 hours after the procedure. The other effects are postprocedural pain and rarely urethral stricture and incontinence [39,40]. Curcumin derivatives also found their place among PSs with the potential to act against prostate cancer. The *in vitro* evaluation indicated that 7-bis(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione had a promising phototoxic effect, resulting in the high reduction of the LNCaP cell line, at the same time having the lowest dark toxicity [41]. New PS delivery mechanisms and nanoparticles, which aim to increase the solubility and bioavailability or even provide additional properties, i.e., allow for PET and optical imaging, inhibit enzymes crucial for cellular redox homeostasis or allow for radiotherapy, are being developed to improve PDT treatment efficacy [42–44]. A set of three novel fluorinated porphyrinoids (porphyrin, chlorin, and isobacteriochlorin) were synthetised, entrapped in self-assembling polyvinylopyrrolidone (PVP), giving the advantage of lower aggregation, thus better perseverance of photophysical properties of these moieties. The formulations were tested against neoplastic, androgen-independent human prostate cell line PC-3, with the highest reduction in cell viability for the formulation containing isobacteriochlorin derivative [42].

Photodynamic methods for bladder cancer

Urothelial carcinoma (UC), or bladder cancer, ranks high at fourth place, constituting 6% of the estimated new cancer cases in the USA only [45]. While a diagnosis of UC mainly relies on recognizing the first symptoms, which might be painless hematuria, dysuria, or urgency, bladder ultrasonography or cross-sectional imaging helps to identify malignancy. However, according to the European Society for Medical Oncology, the unequivocal diagnosing techniques are transurethral resection, biopsy, and histological evaluation or cystoscopy [46].

A significant improvement when using PS is the possibility of performing fluorescent cystoscopy, which up to 30% more accurately detects tumorous lesions in the bladder than a standard white light cystoscopy [47]. Commonly used precursors of PS for this type of PD are 5-aminolevulinic acid (ALA) or its hexyl ester (HAL), the main advantage being the possibility of locating them into the bladder, thus not causing systemic phototoxicity. Both of these compounds depend on the exact mechanism mentioned earlier. ALA or HAL administration results in fluorescent PPIX accumulation in the malignant tissue, which provides a contrasting and phototoxic effect while being excited with appropriate light wavelength [47]. Another molecule researched for UC PD is hypericin, which can emit fluorescence much longer (up to 16 h after the administration) [47].

Regarding diagnosis, PDT can also be successfully used against the UC, providing many advantages such as safety, high selectivity, lower systemic stress compared to conventional chemotherapy, and ease of executing the procedure [48]. The intravesicular HAL solution was administered to 17 patients with high-risk or intermediate non-muscle-invasive UC. A wide-spectrum light source was coupled with a single quartz fibre, and placed in a transurethral irrigation catheter to irradiate the bladder wall. After six months,

52.9% of patients were tumour-free. However, the long-lasting effect (21 months after the procedure) of the absence of the tumour was maintained only in 2 patients [48]. One of the challenges of the PDT of tumours is hypoxia.

For this reason, an oxygen generating MnO_2 nanoparticles (oxygen generating agent) were synthesised. They were then coupled with human serum albumin (HSA) and Chlorin e6 (the PS), forming the HSA-MnO_2-Ce6 complex, which was evaluated in the *in vitro* and *in vivo* studies [49]. The inclusion of an oxygen-generating component in the HSA-MnO_2-Ce6 nanoparticles (NPs) had a significantly enhanced cytotoxic effect while irradiated with the laser, resulting in the lowest cell viability compared to the HSA-Ce6 and HSA-MnO_2 with the second not displaying photoreactivity. Moreover, the HSA-MnO_2-Ce6 NPs provided excellent tumour-targeting ability while not exhibiting apparent accumulation in the mice with normal bladder [49].

Photodynamic methods for the ophthalmology field

The term macular degeneration refers to the age-related variations within this structure. Age-related macular degeneration (AMD) occurs in two forms: exudative (wet) and atrophic (dry) [50]. The dry form occurs significantly more often than the wet form and constitutes 80% of these types of degeneration [51]. Part of the uttermost common symptoms of the AMD wet form implicates blood stroke enclosed by the choroid, which can cause the production of vascular-fibrous membranes located beneath the retina. As a result, the oxygen supply is obstructed in the retina's outer parts, which are situated photoreceptors, leading to their degradation and generating permanent, irreversible retina devastation, termed 'disciform macular degeneration'. The cause of the dry-type macular degeneration is considered progressing thinning and atrophy of the retina close to the macula lutea. The after-effect of the described pathologies is loss of macular function and limited vision in the central part. Qualifying patients with AMD for PDT treatment comprises visual acuity tests with best correction – stereoscopic examination of the fundus with lenses after pupil dilation [52], colour fundus photography, and fluorescein angiography. Among the indications for PDT therapy is the presence of subfoveal choroidal neovascularization developing during AMD, myopia, or histoplasmosis [53].

A contraindication for PDT is a serious pigment epithelial detachment and its atrophy or fracture. After the procedure is performed, hospitalization is not required and can be completed in an outpatient clinic. Side effects of macular degeneration PDT are occasional, and their process is mild. In AMD therapy, Verteporfin is applied as a PS as lyophilized powder under the trade name VISUDYNE® [54]. Furthermore, others report the results of combining PDT with triamcinolone (tc) acetonide injections into the vitreous body. Thirteen patients with an exudative form of AMD, in whom previous PDT had not given expected improvement, underwent this procedure, and they were administered 4 mg tc into the vitreous body after 48–72 hours PDT was performed. Patients were monitored regarding initial side effects in the first and seventh days after the procedure and every three months afterwards. Improved vision has been noted in the case of 76.9 % of treated patients. These results have raised hope in patients with ineffective PDT [55].

Photodynamic methods for gynaecology

There are few gynaecological diseases in which photodynamic methods can be used. It concerns, e.g. *lichen sclerosus*, *leukoplakia vulvae*, malignancies (uterine/cervical, endometrial, ovarian) or endometrial hyperplasia [56]. The vulvar leukoplakia is a non-tumour-like lesion with an underlying chronic inflammatory skin disease of unclear aetiology. Postmenopausal and peri-menopausal females are mainly affected (approximately 3%, compared to children and men 0.1%-0.7%) with vulvar lichen sclerosus (VLS), which often manifests as itching, burning pain, and sexual dysfunction that eventually leads to decreased life quality of the patient [57].

Traditional treatment strategies leave a wide area to improve on, especially in reversing the progression and in the treatment's cosmetic outcome–lasers used to treat VLS are usually abla-

tive; thus, they cause scarring and pain during the procedure [58]. Current methods focus on alleviating or eliminating symptoms, mainly the pruritus of the vulvar regions, by topical application of corticosteroids, hormones, or retinoic acid. However, these solutions are not the best-looking long-term, especially considering the usage of corticosteroids, which may pose a risk of causing irregular skin pigmentation or atrophy; thus, surgical intervention is also a part of VLS treatment with a focus on atypical hyperplasia. [57] Consequently, it is highly recommended that PDT be applied against the VLS. A study on ALA topical application on the lesions included 30 patients who had failed prior conventional treatment.. A gel of 20% ALA concentration was administered on the affected skin and left for three hours, after which the patient was treated with a 635 nm LED light. After a six-month follow-up, patients demonstrated significant improvement in the diminished pruritus and burning pain. Out of 28 patients complaining of itching before ALA-PDT, 25 after the treatment claimed that the symptom disappeared utterly, and the remaining three patients were also relieved considerably [57]. Another study on the group of 70 patients which used ALA-PDT against vulvar leukoplakia was performed using Alasens®, as an aqueous solution of concentration 0.5% topically applied 3–4 hours before the irradiation with an LED light of the 630 nm wavelength [59]. The procedure was performed three times at an interval of 24 hours, and during the irradiation period, it was evaluated based on the decrease of fluorescence of the lesions. The decreased fluorescence intensity in the treated tissue indicates that the accumulated PPIX in the cells converts to photo-reduced forms of "photoproducts," and the photodynamic effect was reached. It shows that the method is effective and constitutes a promising alternative to treat and prevent malignant transformation of vulvar lesions [59]. Another meaningful advantage of the PDT is that it can be successfully used against uterine endometrial cancer while preserving fertility in young females (under 35) [60]. A group of 16 patients with endometrial carcinoma (EC) without myometrial invasion were retrospectively evaluated on the efficacy and overall outcome of PDT. In all cases, a derivative of hematoporphyrin (Photogem®) was used as a PS in the form of an intravenous injection of a dose of 2 mg/kg 48 hours prior to the irradiation with a laser light of 630 nm. A cylindrical optical diffusion fibre delivered the light. The fibre was inserted in the endocervical canal, or the balloon-type diffuser was installed in the endometrial cavity and filled with a standard saline solution.. Twelve of 16 patients initially demonstrated complete remission after the treatment; 4 had recurrence. However, the PDT was performed again, leaving the final positive response rate at 68% (11/16). Seven of the patients attempted pregnancy, and 4 of them had seven successful pregnancies resulting in a total of 6 live births [60]. Considering the statistics of EC incidence the statistics of EC incidence, it is far more common in females of reproductive age (1 in 359) from birth to the age of 49. This retrospective study gives hope that PDT, among the previously mentioned advantages, can also be used as a treatment strategy that preserves fertility [45,60].

Photodynamic methods for head and neck tumors

Tumours of the head and neck are mainly treated surgically. The usage of the PDT in neurosurgery was a breakthrough. In tumour surgical procedures, the surrounding tissues endure damage. PDT treatment diminished the risk of damage to healthy tissue surrounding the lesions. Once the therapy is completed, the risk of developing new poundings on the periphery of the tumour diminishes to a minimum [61].

The autofluorescence phenomenon can also be employed to diagnose neck and head cancers [62]. The discussed method reduces the possibility of making a lapse in choosing, for example, biopsy sampling locations or misdiagnosis. Cell autofluorescence is the result of the UV radiation source and endogenous compounds, for example, aromatic amino acids (tryptophan, tyrosine, phenylalanine) or coenzymes (NADH-nicotinamide adenine dinucleotide, NADPH – reduced NADH form, FAD – flavin adenine dinucleotide, FMN- flavin mononucleotide or else folic acid). The most eminent advantage of autofluorescence diagnostics has become the prospect of lesion imaging, even in patients with severe radiation-induced reactions and in advanced stages of a disease or after radiotherapy [63].

Reported experiments confirmed the described method's usefulness in oral tumour diagnostics. Forty-seven oral cancer lesions, fifty-four precancerous lesions, and thirty-nine normal oral mucosa controls were analyzed, which conducted white light images and VELscope® (Visually Enhanced Lesion Scope; LED Dental Inc., White Rock, B.C.) autofluorescence images taken with a digital camera. After detection and autofluorescence analysis, the average intensity and heterogeneity of the changed areas were calculated. The results of the presented method confirmed that it is entirely sensitive and specific for detecting cancerous lesions [64]. Among the PSs, ALA merits particular attention because it converts to PPIX and selectively accumulates in malignant glioma cells. After that, violet-blue light irradiation of PPIX leads to its excitation, which ultimately leads to the destruction of cancer cells. In head and neck surgery, the autofluorescence phenomenon is used for intraoperative diagnosis to detect remaining glioma cells in boundary surgical areas for radical removal of lesions. Based on research, the described technique called ALA-PD proves its efficacy in increasing the length of patients outliving after surgery [65]. The specific location of head and neck cancers gives rise to frequent relapses. There has been a search for possibly minimally invasive treatment methods [66], and PDT perfectly fulfils this parameter. The evaluation of the treatment of recurrent facial lesions surgical methods and radiotherapy indicates limited medication capabilities, a high risk of complications, and statisti-

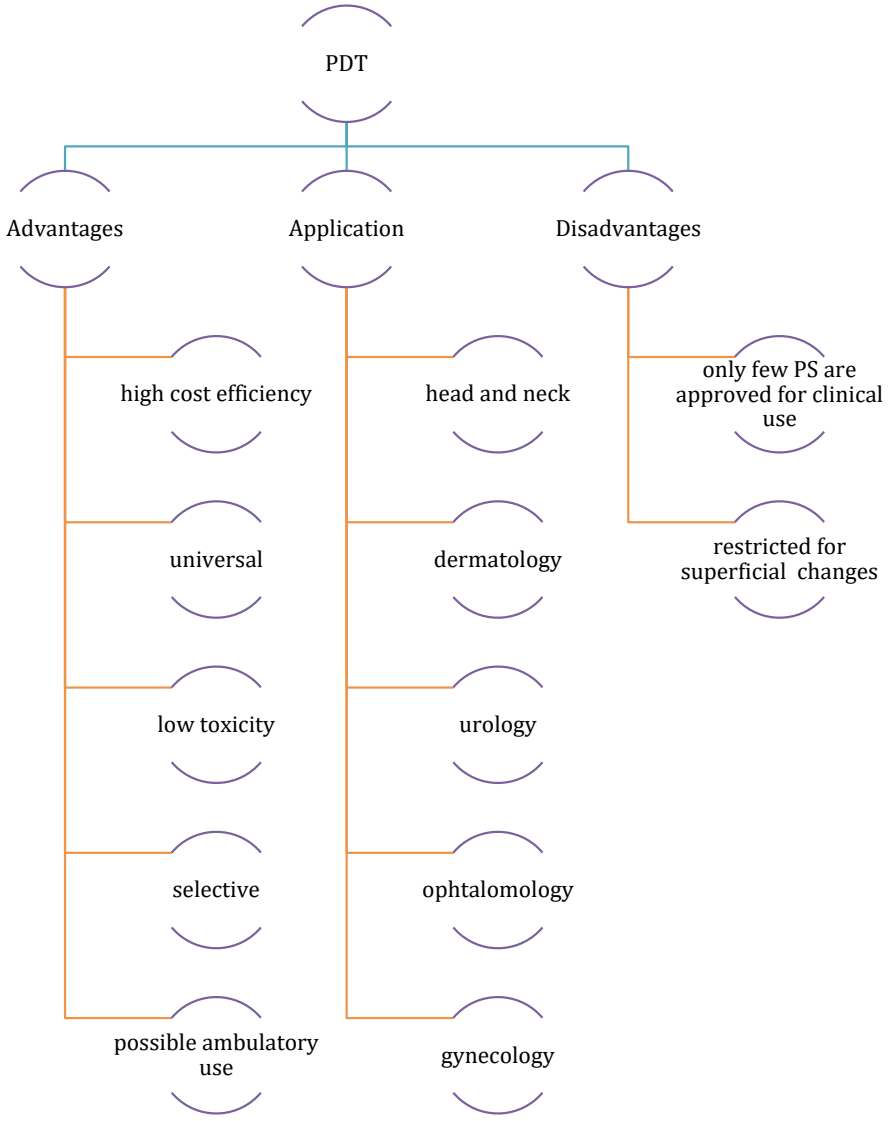

Figure 1. Summary.

cally low patient benefits. In such cases, PDT limits the prevalence of adverse and side effects of radiotherapy and enhances the prospect of complete recovery [67]. Amidst advantages in favour of PDT usage should be mentioned: the possibility of reprised procedures, local effects of PSs, and its selectivity regarding changed tissues [68,69]. The major problem is the accumulation of PSs in the body. In the event of ALA usage, PPIX accumulation occurs due to the overloading of cellular metabolisms of porphyrins. Systemically administered ALA undergoes quick elimination with a terminal half-life of about 1–3 hours [70]. Other PSs used in the treatment of head and neck tumours are HPD – sodium porfimer (Photofrin®); meta-tetra(hydroxyphenyl) chlorin (Foscan®) [71]; boronated porphyrin (BOPP®) [72]; lutetium texaphyrin (Lutex®) [73].

Summary

PDT is considered one of the most intensively developing modern treatment methods. Its main attributes are low cost, universality, low toxicity, high selectivity, and ease of use. Considering the spread of cancerous diseases, PDT development could be beneficial as a complementary approach in modern oncology. Despite the significant successes achieved with photodynamic therapy (PDT) in fighting cancer, there is still room for improvement in several areas. Further development of PDT depends on developing PSs characterised by better selectivity and improving light sources that will deliver the necessary light for therapy to hard-to-reach areas of the human body. The development of PDT holds great hope for combating tumours and improving our quality of life. **Figure 1** shows a summary.

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MANUSCRIPT: Journal of Medical Science publishes Original Articles, Brief Reports, Review articles, Mini-Reviews, Images in Clinical Medicine and The Rationale and Design and Methods of New Studies. From 2014, only articles in English will be considered for publication. They should be organized as follows: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, Conflict of Interest, References and Figure Legends. All manuscripts should be typed in Arial or Times New Roman font and double spaced with a 2,5 cm (1 inch) margin on all sides. They should be saved in DOC, DOCX, ODT, RTF or TXT format. Pages should be numbered consecutively, beginning with the title page.

Ethical Guidelines
Authors should follow the principles outlined in the Declaration of Helsinki of the World Medical Association (www.wma.net). The manuscript should contain a statement that the work has been approved by the relevant institutional review boards or ethics committees and that all human participants gave informed consent to the work. This statement should appear in the Material and Methods section. Identifying information, including patients' names, initials, or hospital numbers, should not be published in written descriptions, illustrations, and pedigrees. Studies involving experiments with animals must be conducted with approval by the local animal care committee and state that their care was in accordance with institution and international guidelines.

Authorship
According to the International Committee on Medical Journal Ethics (ICMJE), an author is defined as one who has made substantial contributions to the conception and development of a manuscript. Authorship should be based on all of the following: 1) substantial contributions to conception and design, data analysis and interpretation; 2) article drafting or critical advice for important intellectual content; and 3) final approval of the version to be published. All other contributors should be listed as acknowledgments. All submissions are expected to comply with the above definition.

Conflict of Interest
The manuscript should contain a conflict of interest statement from each author. Authors should disclose all financial and personal relationships that could influence their work or declare the absence of any conflict of interest. Author's conflict of interest should be included under Acknowledgements section.

Abbreviations
Abbreviations should be defined at first mention, by putting abbreviation between brackets after the full text. Ensure consistency of abbreviations throughout the article. Avoid using them in the title and abstract. Abbreviations may be used in tables and figures if they are defined in the table footnotes and figure legends.

Trade names
For products used in experiments or methods (particularly those referred to by a trade name), give the manufacturer's full name and location (in parentheses). When possible, use generic names of drugs.

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The first page of the manuscript should contain the title of the article, authors' full names without degrees or titles, authors' institutional affiliations including city and country and a running title, not exceeding 40 letters and spaces. The first page should also include the full postal address, e-mail address, and telephone and fax numbers of the corresponding author.

Abstract
The abstract should not exceed 250 words and should be structured into separate sections: Background, Methods, Results and Conclusions. It should concisely state the significant findings without reference to the rest of the paper. The abstract should be followed by a list of 3 to 6 Key words. They should reflect the central topic of the article (avoid words already used in the title).

The following categories of articles can be proposed to the Journal of Medical Science:

ORIGINAL RESEARCH

Original articles: Manuscripts in this category describe the results of original research conducted in the broad area of life science and medicine. The manuscript should be presented in the format of Abstract (250-word limit), Keywords, Introduction, Material and Methods, Results, Discussion, Perspectives, Acknowledgments and References. In the Discussion section, statements regarding the importance and *novelty of the study* should be presented. In addition, the limitations of the study should be articulated. The abstract must be structured and include: Objectives, Material and Methods, Results and Conclusions. Manuscripts cannot exceed 3500 words in length (excluding title page, abstract and references) and contain no more than a combination of 8 tables and/or figures. The number of references should not exceed 45.

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A THOUSAND WORDS ABOUT... is a form of Mini-Reviews. Manuscripts in this category should focus on *latest achievements of life science and medicine.* Manuscripts should be up to 1000 words in length (excluding title page, abstract and references) and contain up to 5 tables and/or figures and up to 25 most relevant references. The number of authors is limited to no more than 3.

OTHER SUBMISSIONS

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Acknowledgements

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

References

All manuscripts should use the 'Vancouver' style for references. References should be numbered consecutively in the order in which they appear in the text **and listed at the end of the paper.** References cited only in Figures/Tables should be listed in the end. Reference citations in the text should be identified by Arabic numbers in square brackets. Some examples:

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

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

Some examples

Standard journal articles

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

Books

Personal author(s)

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

Editor(s) or compiler(s) as authors

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

Chapter in the book

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

TABLES: Tables should be typed on sheets separate from the text (each table on a separate sheet). They should be numbered consecutively with Arabic numerals. Tables should always be cited in text (e.g. table 2) in consecutive numerical order. Each table should include a compulsory, concise explanatory title and an explanatory legend. Footnotes to tables should be typed below the table body and referred to by superscript lowercase letters. No vertical rules should be used. Tables should not duplicate results presented elsewhere in the manuscript (e.g. in figures).

FIGURES: All illustrations, graphs, drawings, or photographs are referred to as figures and must be uploaded as separate files when submitting a manuscript. Figures should be numbered in sequence with Arabic numerals. They should always be cited in text (e.g. figure 3) in consecutive numerical order. Figures for publication must only be submitted in high-resolution TIFF or EPS format (*minimum 300 dpi resolution).* Each figure should be self-explanatory without reference to the text and have a concise but descriptive legend. All symbols and abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure Legends must be included after the reference section of the Main Text.

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SUPPLEMENTARY ONLINE MATERIAL: Authors may submit supplementary material for their articles to be posted in the electronic version of the journal. To be accepted for posting, supplementary materials must be essential to the scientific integrity and excellence of the paper. The supplementary material is subject to the same editorial standards and peer-review procedures as the print publication.

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All manuscripts are reviewed by the Editor-in-Chief or one of the members of the Editorial Board, who may decide to reject the paper or send it for external peer review. Manuscripts accepted for peer review will be blind reviewed by at least two experts in the field. After peer review, the Editor-in-Chief will study the paper together with reviewer comments to make one of the following decisions: accept, accept pending minor revision, accept pending major revision, or reject. Authors will receive comments on the manuscript regardless of the decision. In the event that a manuscript is accepted pending revision, the author will be responsible for completing the revision within 60 days.

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