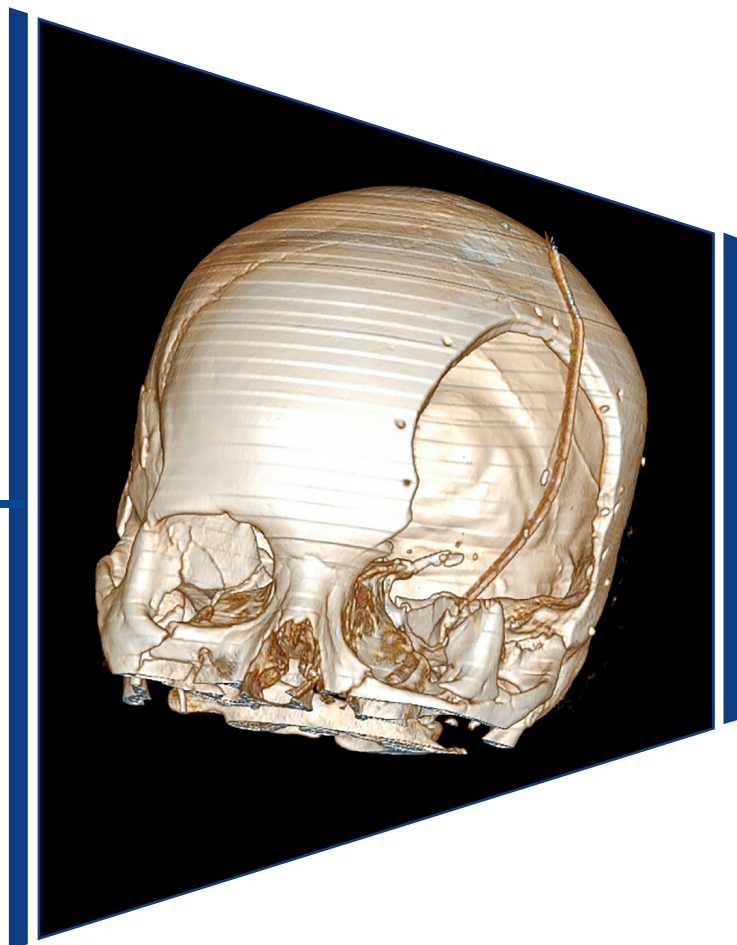




MEDICAL UNIVERSITY OF GDAŃSK

EUROPEAN JOURNAL OF TRANSLATIONAL AND CLINICAL MEDICINE





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Publisher

Medical University of Gdańsk
M. Skłodowskiej-Curie 3 A
80-210 Gdańsk, Poland
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University of Gdańsk

Gdańsk 2023
e-ISSN 2657-3156

Online edition is the
original version of the journal

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Is medicine ready for ChatGPT – why not just ask ChatGPT?

Anthony Rodigin 

Emergency Department, Sutter Delta Medical Center, Antioch, CA, USA

Abstract

As it has often happened in the past, medicine may find itself in captivity of “pioneering and promising” technological trends and fashions. Indeed, while ChatGPT and its progeny may evolve and “become ready for primetime,” we may not – not now, and quite possibly, not ever as an entire species. This brings us back to what we started with: a true philosopher would question humans and not AI. Why do we want AI? Why do we think that we need it? Are we qualified and progressing ethically to first teach AI and then to be able to handle it, so much befriend it, and where is the evidence?

Keywords: ChatGPT • artificial intelligence • AI • medicine • medical education

Citation

Rodigin A. Is medicine ready for ChatGPT – why not just ask ChatGPT? Eur J Transl Clin Med. 2023;6(1):5-8.

DOI: [10.31373/ejtcmm/162647](https://doi.org/10.31373/ejtcmm/162647)

Here we shall concern ourselves, though not overly so, with the potential (or lack thereof) of the artificial intelligence (AI) chatbot named ChatGPT (generative pre-trained transformer) in healthcare, medical education and scientific writing.

Tradition states that medicine is a traditional field. If so, it is not surprising that, just like a kid whose parents for whatever reason forbid all desserts and candy, we may fall for the first available lollipop. With ChatGPT, the prize is a fresh piece of technology, and as such, it seems even more

appealing. So we say to ourselves that we should forcefully and purposefully counterbalance our conservative nature. We cannot allow ourselves to fall behind, we fear. We need to use it. We cannot resist the wave of change, we conclude.

Recently the wind has carried the news that ChatGPT has passed the United States professional licensing exams for lawyers (the Uniform Bar Exam) and physicians (the USMLE) [1-2]. But is anyone shocked? Really? Let's be honest: both of these exams rely on straight memorization (or instantaneous access to the Library of Congress, PubMed and Wikipedia).

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Available online: www.ejtcmm.gumed.edu.pl

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Therefore, why should anyone be astounded that both exams can be passed by a somewhat duller and self-unaware version of C-3PO*? And by the way, what did *you* feel like coming out of that room after taking the USMLE exam?

Instead of pondering what this means for AI and its future, perhaps we ought to focus on our educational future in the context of such exams altogether. Let's face it: to date we have not really understood how human beings learn or how to teach them effectively. For decades we have simply carried on with the tradition of draconian multiple-choice tests as the St. Peter's Gate to fellowship in our craft, social status and income.

But, no doubt (and to no avail), ChatGPT can be the cure for that too! Always polite and courteous, it will write, administer and grade uniform entrance tests, among others. It will then coach the unfortunate failures for next time: "perhaps retinal diseases are not your thing, Annie" as a delicate motherly consolation, "but my analysis of your testing pattern indicates that you have what it takes to be a babysitter extraordinaire."

It is completely obvious why ChatGPT stirs both hype and controversy within society at large. ChatGPT is new, in a lot of ways unprecedented, popular, intriguing and available. ChatGPT now symbolizes the best *and* the worst of civilizational anticipation of AI: grandiose dreams, hopes, plans and fears on the one hand, and all the banalities of commonplace usage on the other. Not so obvious is the question why we are reading so many overly enthusiastic but premature and unfounded reactions, conclusions and expectations, particularly from those thought to be health professionals within their respective job cubicles [3-4].

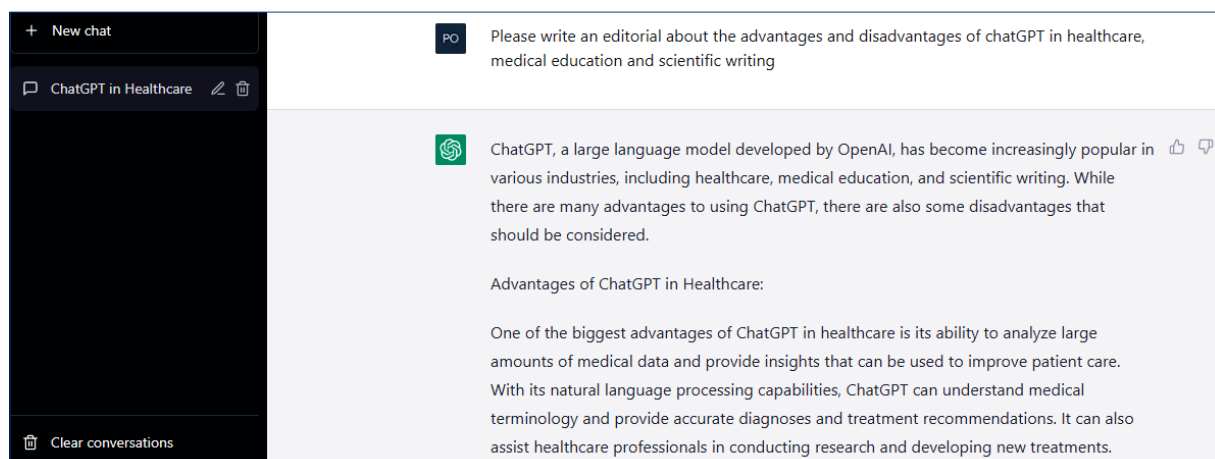
Recently, while my daughter was playing at the park, I had a conversation about all matters AI in medicine with another parent, a professional data scientist who had finished medical school but decided to pursue research. Long story short, he listed numerous instances in his daily work where paradoxically the only force curbing unhindered misplaced excitement and pressure for AI model adaptation coming from physicians, hospital CEOs and other medical clients is his own scientific team. But how many times can one say "no" to a client who is paying you?

Incorrectly formulated clinical questions, horrible data sets, lack of adequate programming and data theory knowledge and unrealistic expectations of AI capabilities have become way too common in medicine already. Should the doctors who only read article abstracts on weekends be trusted with an AI tool? In adapting ChatGPT or a similar program for widespread use, be it medical education or actual clinical care of the patients, whose ethics are we to rely on drawing boundaries if not our own? Finally, the question whether socialized or commercialized medicine will be better at resisting the temptations offered by AI is not a comfortable question to face in the first place. What if neither one?

My own experience with ChatGPT so far is that it is very quick and efficient in retrieving information, saving you hours of eye strain. In my recent "conversation" with ChatGPT in roughly fifteen minutes, we "discussed" a lot of random topics: from bush planes and best local pizza places to sci-fi writer biographies and the gun control debate. I was not invested and did not care all that much about the validity of information I received, therefore I did not verify it. As an aside, ChatGPT miserably fails even rudimentary self-awareness criteria; not sure why anyone would question that – and I don't mean Turing.

Others have reported ChatGPT to generate what we may call apologetic lies, though the medical term *confabulations*, as suggested by my park acquaintance above, is perhaps best. The chatbot apparently makes up stories, data and even full references [5]. As one example, an American physicist and philosopher recently had asked ChatGPT about a real Ukrainian philosopher – and obtained 100% false information including fine details about would-be personal life. After repeating the same question, ChatGPT provided a completely different biography of that same philosopher, also all false [6]. Ironically, being a fantasizing liar is one step closer on the path to self-awareness and "anthropomorphism," not farther.

Naturally, I was unable to resist the temptation to ask ChatGPT to generate this short editorial about its use in healthcare and medical education. Here is its response:



The screenshot shows a chat window with a dark sidebar on the left containing a 'New chat' button and a 'Clear conversations' button. The main area has a light background. A prompt from the user, marked with a 'PO' icon, asks for an editorial on the advantages and disadvantages of ChatGPT in healthcare, medical education, and scientific writing. The response from ChatGPT, marked with its logo, begins by stating its popularity and then lists advantages, starting with its ability to analyze large amounts of medical data.

Prompt: Please write an editorial about the advantages and disadvantages of chatGPT in healthcare, medical education and scientific writing

Response: ChatGPT, a large language model developed by OpenAI, has become increasingly popular in various industries, including healthcare, medical education, and scientific writing. While there are many advantages to using ChatGPT, there are also some disadvantages that should be considered.

Advantages of ChatGPT in Healthcare:

One of the biggest advantages of ChatGPT in healthcare is its ability to analyze large amounts of medical data and provide insights that can be used to improve patient care. With its natural language processing capabilities, ChatGPT can understand medical terminology and provide accurate diagnoses and treatment recommendations. It can also assist healthcare professionals in conducting research and developing new treatments.

Prompt for the Chat-GPT generated essay and beginning of the response. [See full text here.](#)

Without dwelling too much on what ChatGPT wrote ([the full text is here](#)), I cannot help but to ponder the following. First, what “insights” and “personalized recommendations” can be provided by a polite confabulator? Second, what more “important tasks” other than formulating and spelling out own conclusions should medical researchers be freed up to focus on? Third, as accurately self-disclosed, ChatGPT is in fact a tool and therefore it is subject to every bias of an AI system and the data quality provided to it. But as Capt. Pete “Maverick” Mitchell has repeatedly told us: “it’s not the plane, it’s the pilot” [7]. Last, all the wishy-washy talk about “improving patient care” and “enhancing medical education” really sounds like a poorly written medical school admission essay (no, I am not giving anyone any ideas!) that also discusses “helping people” and “serving humanity.” Might be true, but way too cliché.

In all honesty, any “analysis” of ChatGPT at this stage, even omitting the fact that it is a prototype, restricted in its scope and resources and is under development, will tend to place it into one of three ponds: Chat as a rather negative and evil, Chat as amazing and a panacea, or Chat as something in between, depending on this and that [5, 8]. The vast majority of opinions fall in the latter. While quite possibly correct, they are also very much predictable and as such, very unilluminating and boring. Citing the inevitability of AI development (but wait, that development does not just happen on its own – we’re the ones driving it!), and ChatGPT as a “game changer”, most will conclude with a limited cautious optimism toward implementation [9].

More introspective and even wiser articles would focus on ourselves, the people, and not the ChatGPT platform. Indeed, while ChatGPT and its progeny may evolve and “become ready for primetime,” we may not – not now, and quite possibly, not ever as an entire species [10]. This brings us back to what we started with: a true philosopher would question humans and not AI. Why do we want AI? Why do we think that we need it? Are we qualified and progressing ethically to first teach AI and then to be able to handle it, so much befriend it, and where is the evidence?

Thanks to multiple science fiction writers and film producers, thoughts of a benign friendly AI are by now deeply instilled in our minds and in our collective subconscious. From David 8 in “Prometheus” and Data in “Star Trek” to Sonny in “I, Robot” and Andrew Martin in “Bicentennial Man,” we seem to long for an autonomous, self-aware, amiable, but artificial companion. In the medical profession, we of course all want to hang with the benevolent and clinically omnipotent Baymax pet. Is this just another manifestation of our weaknesses and insecurities? Or is it yet another technological push in the hope to one day achieve immortality?

My final point is that while we can debate what ChatGPT may mean for and bring to medical student education, we should not forget what Dr. Google has already accomplished for our patients’ anxiety levels. Because it may very well turn out that while we are preoccupied with Chat’s future version 17.0 and its improvements, our patients will again be way ahead of us in Chat-diagnosing and Chat-prescribing.

As it has often happened in the past, medicine may find itself in captivity of “pioneering and promising” technological trends and fashions. In 2003 as a 2nd year medical student I succumbed to such pressure and purchased a then-state of the art Palm Pilot... and had never even opened the box. I wonder what Data and Baymax would say about that. By the way, can I still get a refund?

Disclaimer

All views are only of the author and not any affiliated organizations.

Funding

None.

Conflicts of interest

There is no conflict of interest in this project.

*The C-3PO is the iconic humanoid robot character from the “Star Wars” films.

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Persistence of long-term insulin independence after islet transplantation and two subsequent pregnancies

Sarah Gondek , Mateusz Ogledzinski , William Lin , Kamila Milejczyk , Braden Juengel , Lisa Potter , Piotr J. Bachul , Lindsay Basto , Laurencia Perea , Ling-Jia Wang , Martin Tibudan , Zuzanna Witkowska , Rolf Barth , John Fung , Piotr Witkowski 

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Abstract

Pregnancy increases metabolic demand for insulin and may lead to the exhaustion of intraportally transplanted islets and post-gestational hyperglycemia. To prevent these complications, we implemented preemptive insulin supplementation during two subsequent pregnancies in an insulin-independent islet transplant recipient. This strategy resulted in optimal blood glucose control during the pregnancies, the preservation of the optimal islet graft function and the postpartum maintenance of long-term insulin independence.

Keywords: pregnancy • insulin • islet transplantation

Citation

Gondek S, Ogledzinski M, Lin W, Milejczyk K, Juengel B, Potter L, Bachul PJ, Basto L, Perea L, Wang L, Tibudan M, Witkowska Z, Barth R, Fung J, Witkowski P. Persistence of long-term insulin independence after islet transplantation and two subsequent pregnancies. *Eur J Transl Clin Med.* 2023;6(1):9-13.

DOI: [10.31373/ejtcml/166178](https://doi.org/10.31373/ejtcml/166178)

List of abbreviations

- BG – blood glucose;
- T1DM – Type 1 Diabetes Mellitus;
- IRB – Institutional Review Board;
- ITx – islet transplantation;
- IEQ – islet equivalent; CXCR1/2 – chemokine receptor 1 and 2

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Available online: www.ejtcml.gumed.edu.pl

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Introduction

Blood glucose (BG) in the narrow physiological range is essential for a human body's homeostasis. BG is controlled by pancreatic islets spread within the pancreatic gland located in the abdominal cavity. Each islet is a micro-organ, having a defined anatomical structure with its vasculature and neural network [1]. Although it is a single anatomical and physiological endocrine unit, the function of each islet is integrated very well with the function of the remaining islets submerged in the exocrine tissue of the pancreas. In normal conditions, only some islets actively regulate BG via secreting an appropriate amount of insulin and glucagon. The remaining islets are dormant and constitute a functional reserve activated only when necessary to respond to higher metabolic demand [2]. Such system allows them to adapt to metabolic demand and protects islets from constant metabolic stress and subsequent failure from exhaustion.

Islet transplantation (ITx) is a minimally-invasive alternative to whole pancreas transplantation for patients with poorly-controlled type 1 diabetes mellitus (T1DM). Islets are retrieved from the deceased donor pancreas in a laboratory and infused intraportally suspended in a transplant media via a small catheter placed percutaneously and transhepatic by an interventional radiologist under local anesthesia. During a single transplant procedure, the patient receives a limited islet mass as current technology allows to retrieve, on average, only 30-60% of the 1 million islets present in the human pancreas. Much less than 75% of those infused islets engraft into the liver and resume metabolic activity [3-5]. Patients with T1DM, on average, require at least two islet transplantations to obtain ~60% of the islet mass physiologically present in a healthy pancreas. Such islet mass is usually able to provide a sufficient amount of insulin for the patient to achieve and maintain insulin independence. However, under such conditions, an islet graft does not provide a functional islet reserve the same way a native pancreas or a whole pancreas transplant does.

Therefore, any extraordinary metabolic demand may lead to islet graft stress. If it persists over an extended period, it may result in islet graft exhaustion, failure and recurrence of diabetes. For this reason, physiologic pregnancy with progressing metabolic demand cause exhaustion of intraportally transplanted islets and recurrent diabetes, as observed previously [6-8]. Importantly, suboptimal blood glucose control, which may occur during pregnancy in islet transplant recipients, increases the chance of complications and birth defects, as presented previously [8]. To prevent these complications, preemptive insulin supplementation during the pregnancy in an islet transplant recipient was implemented and presented as a case report [9]. It resulted in the preservation of islet graft function and

optimal blood glucose control without complications to the mother and the baby [9]. In our study, we aimed to verify the efficacy of that approach. We confirmed its utility in the long-term preservation of islet graft function after two subsequent pregnancies in our patient.

The case

The patient was a 29-year-old female with T1DM, hypoglycemia unawareness and frequent severe hypoglycemic episodes, despite advanced diabetic medical treatment. Her body weight was 63.6 kg, and her BMI was 23.7. She developed long-term insulin independence with HbA1c < 5.8% after a single intraportal ITx (islet mass 635,554 islet equivalents (IEQ); and 9,999 IEQ/kg of body weight). Anti-thymocyte globulin (Thymoglobulin, Sanofi, US), along with CXCR1/2 inhibitor (Reparixin, Dompe, Italy), was used for induction and tacrolimus with mycophenolate were used for maintenance immunosuppression. Several months before conception, mycophenolate was replaced with azathioprine 125 mg to eliminate its teratogenic effect while decreasing the tacrolimus target trough level to 4-6 ng/ml. The patient became pregnant twice, 5 years and 7.5 years after her islet transplantation. At the conception of her first pregnancy, she was 34 years old, with a BMI of 25 and 66 kg of body weight. During the first pregnancy, the patient pre-emptively supplemented with insulin doses of 5-10 units per day in the first trimester, 15-45 units per day in the second, and 35-70 units per day in the third trimester. She supplemented with up to 35 units per day during the second pregnancy to target fasting blood glucose < 95 mg/dl.

Postpartum, the patient was successfully weaned off insulin maintaining optimal blood glucose control with HbA1c < 5.7%. Islet graft function before and after the pregnancy remained optimal, as reflected by fasting blood glucose, C-peptide, HbA1c, and BETA-2 index above 17 (Figure 1A)[10]. BETA-2 score is a composite index calculated based on fasting C-peptide, fasting blood glucose, HbA1c, and insulin used. BETA-2 was validated to provide information about islet graft function after allo- and auto-transplantation [10-11]. BETA-2 score > 17 is correlated with optimal islet graft function, whereas declining values < 17 correspond to worsening islet graft function. BETA-2 of zero indicates complete graft failure and lack of C-peptide detection in the blood. A mixed meal tolerance test confirmed optimal and stable islet function with comparable C-peptide secretion and peak blood glucose levels < 130mg/dl during the entire 8.5-year follow-up (Figure 1B). Of note, BETA-2 during pregnancy dropped below the insulin independence threshold of 17 due to the pre-emptive insulin supplementation for increased metabolic demand rather than true islet dysfunction.

Both newborns were premature and delivered at 34 weeks via emergent C-sections due to preeclampsia, even though the recommended prophylactic aspirin dose of 81 mg was

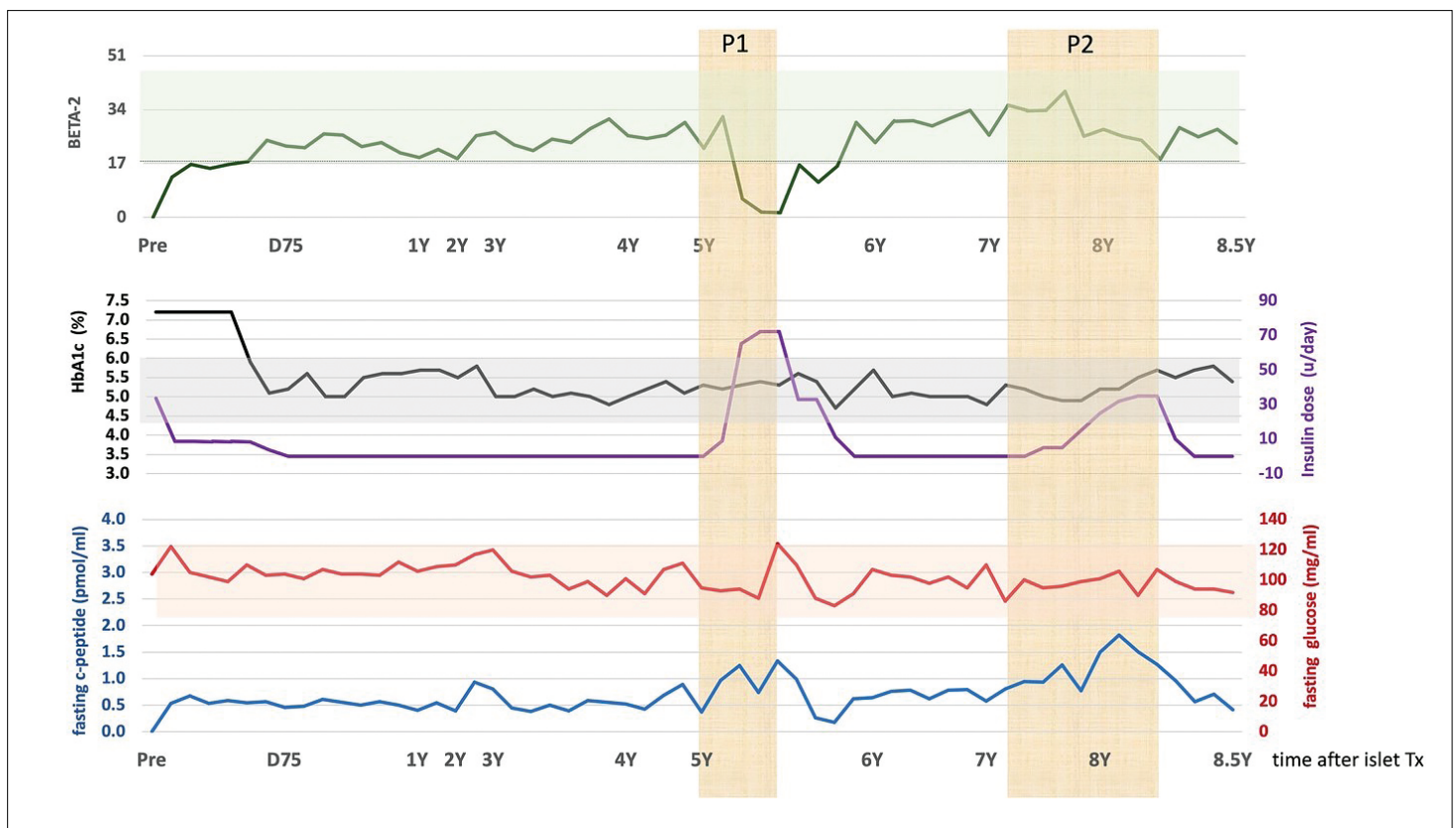


Figure 1A. Islet graft function over time during the follow-up based on BETA-2, HbA1c, fasting C-peptide and blood glucose and insulin requirements. P1 and P2 yellow areas represent period of first and second pregnancy, respectively.

Islet graft function remained optimal with a BETA-2 score over the threshold of 17 for insulin independence (green line in the light green zone), besides a period of pre-emptive insulin supplementation during the first pregnancy (P1).

HbA1c dropped after the islet transplant and remained below 6.0% during the entire follow-up (black line in gray zone).

Purple line represents doses of insulin supplementation. Insulin was given only pre-emptive during both pregnancies.

Fasting blood glucose remained in optimal range below 120 mg/ml during the entire follow up (red line in the light red zone)

Fasting c-peptide also remained in the physiological range of 0.3- 1.7 pmol/ml during the entire follow-up (blue line).

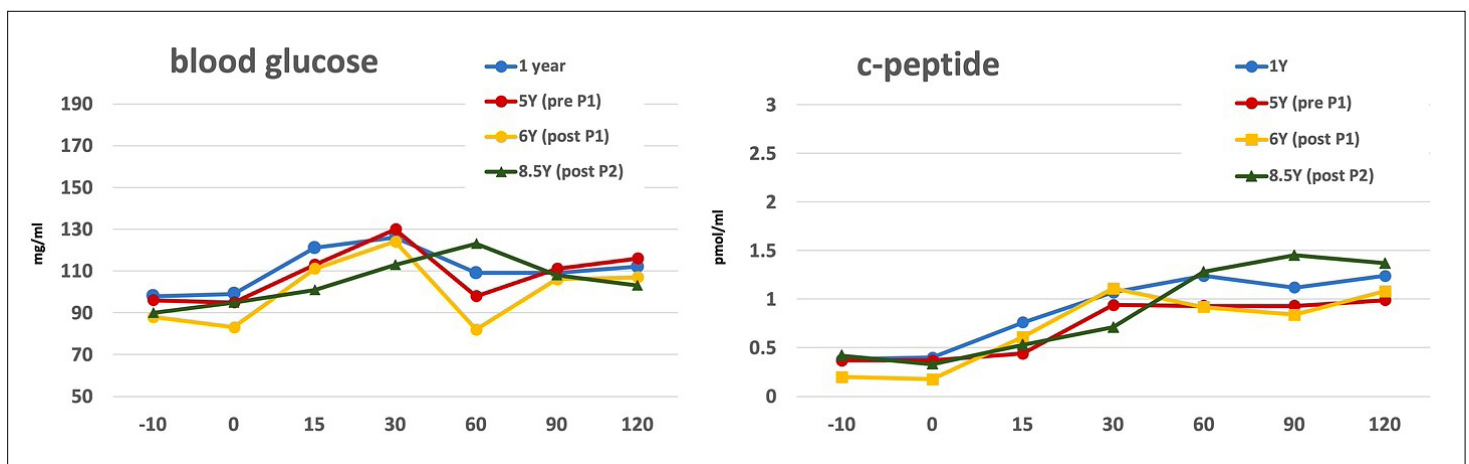


Figure 1B. Islet graft function measured based on the mixed meal tolerance test

Islet graft function remained stable and optimal, reflected by fasting blood glucose below 100 mg/ml and peak glucose below 130 mg/ml.

Area under the curve for serum c-peptide did not change significantly between 1 year and 8.5 years after the islet transplant:

0.45 min*pmol/ml vs 0.42 min*pmol/ml, respectively ($p > 0.05$)

administered daily following the 12th week of pregnancy [12]. Unfortunately, the first newborn died due to necrotizing enterocolitis, a known complication after premature delivery (16% mortality). The second child has been nursed and developing appropriately at eight months after their birth, while the patient remains insulin independent with an HbA1c of 5.6%.

Discussion

We found only a single case report presenting a successful pregnancy and the preservation of optimal islet graft function after pre-emptive insulin supplementation during the pregnancy in insulin-independent patients [9]. In contrast to our study, the lack of insulin supplementation in otherwise insulin-independent patients reported in one of the previous studies resulted in suboptimal blood glucose control (elevation of fasting blood glucose) during the pregnancy, birth defect imperforated anus and postpartum gradual islet graft loss and need for insulin therapy [8]. As no signs of immunologic rejection or patient noncompliance to immunosuppression were reported, islet graft dysfunction during and after pregnancy was most likely related to increased metabolic demand during the pregnancy leading to chronic metabolic stress and exhaustion of sub-physiologic islet graft mass [8]. Pre-emptive supplementation of insulin to prevent metabolic stress of islet graft during the pregnancy was reported effective previously, and now we replicated the results confirming its utility in our patient [9].

Despite the absence of known risk factors for preeclampsia (hypertension, elevated creatinine, obesity, and infection), our patient required maintenance immunosuppression, which might have put her at higher risk for preeclampsia. Pre-eclampsia is reported in as 20-30% in organ transplant recipients, compared to 5-6% in the general population [13]. It is possible that immunosuppression medications directly affected maternal microcirculation leading to a mismatch with the fetoplacental demand leading to preeclampsia despite a lack of clear tacrolimus nephrotoxicity as serum creatine remained in the range of 0.9-0.97 mg/ml prior and 8.5 years after the ITx [13].

Our results support the routine implementation of pre-emptive insulin support during pregnancy, however the dose should be carefully monitored and adjusted to prevent hypoglycemic episodes. Our report also underscores the high-risk nature of pregnancy in islet transplant recipients and the need for close clinical monitoring for risk factors and pre-eclampsia.

Conclusions

In light of limited experience and the growing number of patients successfully cured of T1DM with islet transplantation, our results provide important data guiding optimal patient management during pregnancy for the benefit of the mother and the baby and the preservation of the islet graft function.

Acknowledgments

The study was supported by the Dompe Farmaceutici S.p.A. The authors would like to acknowledge the generosity and support of Dr. Martin Jendrisak and the entire team of the Gift of Hope Organ & Tissue Donor Network in Chicago for providing the human pancreas for transplantation. We also acknowledge support from the NIDDK P30 DK020595 and the Kovler Family Fund.

Conflicts of Interest

PW served as a consultant to the Dompe Farmaceutici regarding a liver transplantation study and received a grant/funding to conduct two studies about islet transplantation. The remaining authors declare no conflict of interest.

Funding

None.

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Clinical and demographic features of acutely hospitalised schizophrenia patients according to *Toxoplasma gondii* serostatus

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Abstract

Background: Few data exist concerning the clinical correlates of *Toxoplasma gondii* infection in persons with schizophrenia. The aim of this study was to investigate the correlation between toxoplasmosis and schizophrenia regarding the quality of life, symptoms and course of hospitalisation in patients with schizophrenia. **Methods:** Acutely hospitalised patients (n = 67) were examined twice during their hospital stay. Schizophrenia psychopathology, quality of life, extrapyramidal symptoms and *T. gondii* antibody titres were assessed upon admission and at discharge. **Results:** Toxo-IgG (+) patients (59.7%) were older, less educated, more obese and less eager to undertake psychotherapy. Female gender and higher fertility were dominant in this group with abnormal involuntary movements more commonly observed. Lower antipsychotic drug doses and monotherapy were used more frequently for Toxo-IgG (+) patients. Lower education (OR 2.41, 95% CI 1.21-4.79) was the most important factor associated with higher likelihood of IgG seropositivity. High levels of Toxo-IgM antibodies correlated with lower quality of life (r = -0.37; p = 0.02) and more severe positive (r = 0.40; p = 0.01) and focal (r = 0.32; p = 0.04) schizophrenia symptoms. **Conclusions:** Toxoplasmosis is more common in older, obese women with lower education. Recent infection is linked to more severe schizophrenia symptoms. Patients with toxoplasmosis history were given less medication.

Keywords: quality of life • infection • tardive dyskinesia • toxoplasmosis • psychotic disorders

Citation

Grabowski J, Waszak P, Przybylak M, Bidzan L. Clinical and demographic features of acutely hospitalised schizophrenia patients according to *Toxoplasma gondii* serostatus. Eur J Transl Clin Med. 2023;6(1):14-24.

DOI: [10.31373/ejtcmm/162759](https://doi.org/10.31373/ejtcmm/162759)

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Introduction

A possible link between schizophrenia and toxoplasmosis has been the subject of study for the last 70 years [1-3]. It was suggested that infection by the protozoa *Toxoplasma gondii* may have an etiological role in the development of schizophrenia [4]. Epidemiological observations showed that the rates of *T. gondii* antibodies in people with schizophrenia are 2.7 higher than in healthy controls [5], with an odds ratio exceeding that for genetic or any other environmental factors identified so far.

In this study we aimed to provide a different approach and methodology than studies performed hitherto about the correlation between toxoplasmosis and schizophrenia. Our first aim was to measure the quality of life and extrapyramidal symptoms (EPS) in *T. gondii* seropositive patients with schizophrenia. The second aim was to measure the symptomatology of schizophrenia in patients over time with relation to toxoplasma IgG and IgM antibody titres.

Material and methods

This is a case series study. Only schizophrenia patients (cases) made up the sample in this study and the exposure to *T. gondii* was evaluated retrospectively.

Subjects in the study were inpatients of Regional Psychiatric Hospital in Gdańsk (Poland) hospitalised due to exacerbation of schizophrenia. With 111 patients enrolled, final analysis was possible for a study sample that consisted of 67 patients. The study protocol and the informed consent form were approved by the Regional Ethics Committee. All of participants in this study voluntarily gave their informed consent. Main inclusion criteria were age 18-65 and a hospitalisation due to exacerbation of schizophrenia diagnosed according to the DSM-V (Diagnostic and Statistical Manual of Mental Disorders). The following were the exclusion criteria: lack of consent or refusal to participate in the study at any time during the observation, involuntary hospitalisation, co-existence of another axis I disorder according to the DSM-V, co-existing serious somatic illness (uncontrolled cardio-vascular disorders, uncontrolled respiratory disease, kidney or liver failure, recent stroke or heart attack etc.), features or a history of organic lesion of the central nervous system (CNS), except those clearly related to *T. gondii* infection. Subjects were enrolled upon hospital admission with procedures performed twice during the hospitalisation: within three days of admission and up to 7 days prior to discharge from hospital.

T. gondii antibodies' level was measured by enzyme-linked immune-sorbent assay (ELISA) test (positivity index > 1.0 for IgM and > 0.130 for IgG). We used the electrochemiluminescence immunoassay (ECLIA) on the Cobas e analyzer (Roche, Switzerland). Samples for IgM antibodies were taken upon admission and at discharge (to exclude a possible window period), while samples for IgG evaluation

only on admission to the hospital. Patients were divided into two main groups based on IgG seropositivity with additional calculations made for IgM (+) and IgM (-) subjects.

PANSS (Positive and Negative Syndrome Scale) [6] results compared between the groups and subgroups were analysed as a whole, with classical distinction to positive (P1-P7), negative (N1-N7) and general (G1-G16) symptoms, PANSS focal symptoms [7], as well as according to the PANSS five-factor model (PANSS-FCTcr) [8]. Assessment of psychopathological improvement was made according to Leucht's 25% and 50% PANSS reduction criteria [9]. The schizophrenia remission rate was compared with the use of three different remission concepts [7-8, 10].

Extrapyramidal symptoms were assessed using the modified Simpson-Angus Scale (mSAS) [11] and the Abnormal Involuntary Movement Scale (AIMS) [12]. Additionally, the presence of extrapyramidal symptoms (EPS) during the hospitalisation was acknowledged by reaching one of mSAS thresholds (≥ 0.3 and ≥ 0.65) [13] or by the necessity to administer any drug to alleviate the EPS.

Quality of life was assessed using the Personal and Social Performance Scale (PSP) [14]. Doses of medication at discharge were recalculated into chlorpromazine equivalents (CPZE) [15-16] to enable comparison between different drugs. All researchers were certified to use the study scales. The researchers did not participate in the direct treatment process of the participants, therefore they were not aware of the subjects' serostatus.

Data from the structured interview including the socio-demographic status, course of illness to date, history of pharmacotherapy side-effects and compliance was gathered as a source for comparison of study groups. Smoking status was determined to exclude its potential impact on negative and extrapyramidal symptoms according to the self-medication hypothesis, as well as on medication doses by inducing the CYP4501A2 metabolism pathway. Patients' characteristics are shown in Table 1.

Relative proportions were calculated with a confidence interval of 95%. Possible associations were identified using the Chi-Square and Fisher's exact statistical tests, as well as the t-Student (or Mann-Whitney U test). Verification of whether the sample came from a normally distributed population was made using the Shapiro-Wilk test while Levene's (Brown-Forsythe) test was used to assess the equality of variances for a variable calculated for two or more groups. Analysis of the differences among group means was made with the help of ANOVA (Analysis of variance) or Kruskal-Wallis test. The Spearman correlation was used to analyse the correlation between continuous variables. Stepwise multivariate logistic regression analysis was performed with Somers' D and Kolmogorov-Smirnov statistics. Odds ratios were also calculated. Significance level was $p = 0.05$. Statistical analysis was performed using the Statistica software (StatSoft Co, Tulsa, OK; USA), with the exception of power analysis for which we used ClinCalc (ClinCalc LLC; available at <https://clincalc.com/>).

Table 1. Baseline patient characteristics

	Male [N = 44]	Female [N = 23]	P-value
Age – [years] Mean (SD)	39.93 (± 10.70)	47.22 (± 13.23)	0.02
BMI – [kg/m2] Mean (SD)	26.61 (± 5.11)	29.84 (± 16.76)	NS
Comorbidities	20 (45.45%)	12 (52.17%)	NS
Schizophrenia			
Illness duration [years] – Mean (SD)	14.32 (± 9.41)	18.30 (± 20.24)	NS
Past hospitalizations [number] – Mean (SD)	14.14 (± 14.79)	10.83 (± 20.47)	NS
Current hospitalisation length [days] – Mean (SD)	48.66 (± 30.39)	43.48 (± 26.28)	NS
Social situation			
Education			NS
University	5 (11.36%)	4 (17.39%)	
High school	13 (29.55%)	9 (39.13%)	
Vocational school	8 (18.18%)	3 (13.04%)	
Primary	18 (40.91%)	6 (26.09%)	
Living conditions			NS
alone	7 (15.91%)	5 (21.74%)	
with family of origin	28 (63.64%)	8 (34.78%)	
with the procreative family	6 (13.64%)	10 (43.48%)	
institutionalized	2 (4.55%)	0	
Income			NS
work	4 (9.09%)	5 (21.74%)	
unemployment	9 (20.45%)	7 (30.43%)	
social benefits	31 (70.45%)	11 (47.83%)	
Treatment			
Antipsychotic drugs			NS
mix (+LAI)	13 (29.55%)	7 (30.43%)	
SGA (+LAI)	2 (4.55%)	2 (8.70%)	
FGA (+LAI)	3 (6.82%)	3 (13.04%)	NS
SGA	16 (36.36%)	8 (34.78%)	
mix	7 (15.91%)	2 (8.7%)	
FGA	3 (6.82%)	1 (4.35%)	
CPZE equivalents (mg)	899.32 (± 613.33)	537.91 (± 336.92)	0.01
Clozapine users	14 (31.82%)	4 (17.39%)	NS
EPS correcting medications	18 (40.91%)	7 (30.43%)	NS

BMI – body mass index, CPZE – chlorpromazine equivalent doses, EPS – extrapyramidal symptoms, FGS – first generation antipsychotics, LAI – long-acting injectable antipsychotic drugs, NS – not significant, SD – standard deviation, SGA – second generation antipsychotics

Results

44 men and 23 women were assessed. Women were slightly older than men (47.22 vs. 39.93; $p = 0.02$) and differed from them in lower doses of antipsychotic drugs (537.91 vs. 899.32 CPZE equivalents; $p = 0.01$).

Characteristics of subjects with a history of latent toxoplasmosis infection (59.7% with positive Toxo-IgG antibodies) differ from the Toxo-IgG (-) group in some aspects (Table 2). No significant differences were found between Toxo-IgG (+) and Toxo-IgG (-) subjects in terms of current course of the disease, level of cooperation in the treatment or used drugs of abuse. The course of current hospitalisation did not differ regarding the drug groups (classical/atypical/long acting injectables) used or the percentage of patients who responded to treatment or achieved remission of schizophrenia symptoms.

However, Toxo-IgG (+) patients received mean lower doses of antipsychotics (CPZE mg 671.2) and were more often treated with monotherapy than the Toxo-IgG (-) patients (37.5% vs. 14.8%).

We observed a significantly higher intensity of involuntary movements in the Toxo-IgG (+) group regarding both AIMS-Total Severity (AIMS-TS) and AIMS-Global Severity (AIMS-GS) subscales. Tardive dyskinesia threshold of AIMS scale [17] was reached more frequently by patients with Toxo-IgG (+) than those with Toxo-IgG (-) but the results were not statistically significant (12.5% vs. 0.0%; $p = 0.0562$). Significant reduction in the extrapyramidal symptoms measured with the mSAS scale was more frequent in the anti-toxoplasmosis IgG (+) group ($p < 0.05$). The results of other scales and subscales were not significantly different between the groups studied.

Table 2. Sample characteristics based on Toxo-Ig status

		Toxo-IgG (+)	Toxo-IgG (-)	P-value	Toxo-IgM (-)	Toxo-IgM (+)	P-value
		N = 40	N = 27		N = 63	N = 4	
	Age - Mean (SD)	45.9 (12.5)	37.3 (9.5)	< 0.01	42.5 (12.0)	41.8 (14.1)	0.9288
	Length of hospital stay (days) - Mean (SD)	49.0 (31.4)	43.8 (25.1)	0.65	46.8 (28.4)	48.8 (42.1)	0.8283
Gender	Female	19 (47.5%)	4 (14.8%)	< 0.01	20 (31.8%)	3 (75.0%)	0.0773
	Male	21 (52.5%)	23 (85.2%)		43 (68.3%)	1 (25.0%)	
	Number of children - Mean (SD)	1.0 (1.4)	0.3 (0.7)	< 0.05	0.7 (\pm 1.3)	0.5 (1.0)	0.8070
Education	Primary	16 (40.0%)	8 (29.6%)		23 (36.5%)	1 (25.0%)	0.7896
	Vocational school	9 (22.5%)	2 (7.4%)		11 (17.5%)	0	
	High school	13 (32.5%)	10 (37.0%)		20 (31.7%)	2 (50.0%)	
	University	2 (5.0%)	7 (25.9%)		8 (12.7%)	1 (25.0%)	
	Age at onset (years) - Mean (SD)	29.8 (11.4)	25.1 (7.4)	0.117	27.8 (\pm 10.0)	29.7 (13.2)	0.8884
	Illness duration (years) - Mean (SD)	16.3 (10.1)	11.6 (7.5)	< 0.05	14.3 (9.4)	37.2 (43.2)	0.0000
	Number of hospitalisations - Mean (SD)	11.4 (14.0)	12.1 (11.6)	0.354	13.0 (16.9)	13.3 (18.3)	0.6366
	Current hospitalisation due to noncompliance	33 (84.6%)	22 (81.5%)	0.737	43 (68.3%)	3 (75.0%)	0.0002
	Body Mass Index (kg/m ²) - Mean (SD)	27.5 (6.3)	25.4 (4.1)	0.279	28.0 (10.9)	23.0 (1.2)	0.1318
	Fasting blood sugar (mg%) - Mean (SD)	95.5 (17.7)	86.9 (17.6)	< 0.05	92.6 (18.2)	88.0 (11.7)	0.5828
	Overweight	22 (55.0%)	13 (48.1%)	0.5818	35 (55.6%)	0	0.0310
	Obesity	15 (37.5%)	2 (7.4%)	< 0.01	17 (27.0%)	0	0.2291

Table 2. continued

		Toxo-IgG (+)	Toxo-IgG (-)	P-value	Toxo-IgM (-)	Toxo-IgM (+)	P-value
	History of psychotherapeutic treatment	14 (35.9%)	19 (70.4%)	< 0.01	31 (49.2%)	3 (75.0%)	0.3170
	Current smokers	26 (65.0%)	19 (70.4%)	0.6461	44 (69.8%)	1 (25.0%)	0.0641
	Cigarettes smoked daily – Mean (SD)	24.0 (12.2)	25.9 (21.6)	0.739	50.7 (38.9)	83.2 (35.5)	0.1466
	CPZE (mg) – Mean (SD)	671.2 (516.2)	929.4 (594.2)	< 0.05	774.2 (548.4)	791.5 (816.8)	0.7497
Discharge therapy	Polytherapy	25 (62.5%)	23 (85.2%)	< 0.05	45 (71.4%)	3 (75.0%)	0.8764
	Monotherapy	15 (37.5%)	4 (14.8%)		18 (28.6%)	1 (25.0%)	
Admission	PANSS Total Score – Mean (SD)	116.1 (12.7)	116.2 (14.9)	0.9287	115.6 (13.4)	124.3 (13.7)	0.3479
	Focal PANSS – Mean (SD)	32.7 (5.8)	33.4 (5.4)	0.858	32.8 (5.6)	35.8 (6.2)	0.3616
	PANSS Positive Factor – Mean (SD)	17.2 (3.8)	17.1 (4.0)	0.9694	16.9 (3.8)	20.5 (1.3)	0.0428
	PANSS Negative Factor – Mean (SD)	20.8 (6.0)	21.3 (5.4)	0.5353	21.0 (5.6)	20.3 (8.7)	0.8283
	PANSS Disorganised Factor – Mean (SD)	13.2 (2.4)	12.9 (2.6)	0.5022	13.1 (2.6)	13.0 (0.8)	0.5828
	PANSS Excited Factor – Mean (SD)	18.0 (5.6)	17.0 (6.2)	0.5145	17.3 (5.8)	22.5 (5.3)	0.0781
	PANSS Depressed Factor – Mean (SD)	8.6 (3.2)	9.6 (3.4)	0.2473	9.1 (3.3)	7.8 (3.8)	0.6366
	mSAS – mean (SD)	0.3 (0.4)	0.3 (0.3)	0.6047	3.1 (3.4)	2.3 (4.5)	0.3479
	AIMS TS – mean (SD)	1.0 (2.0)	0.1 (0.3)	< 0.05	0.3 (0.7)	0.5 (1.0)	0.7887
	Tardive dyskinesia	4 (10.0%)	0 (0.0%)	0.0902	3 (4.76%)	1 (25.0%)	0.0976
	PSP	26.1 (10.4)	30.3 (13.7)	0.3442	28.3 (12.0)	19.0 (6.5)	0.0500
	PANSS Total Score – Mean (SD)	74.5 (15.4)	73.7 (14.1)	0.8379	74.5 (14.9)\	69.3 (13.7)	0.5828
	Focal PANSS – Mean (SD)	21.0 (5.9)	21.1 (4.9)	0.7445	21.2 (5.4)	19.0 (6.8)	0.6005
	PANSS Positive Factor – Mean (SD)	9.0 (3.3)	9.1 (3.2)	0.8882	9.1 (3.3)	7.5 (2.4)	0.3756
	PANSS Negative Factor – Mean (SD)	17.4 (5.5)	16.9 (4.6)	0.8831	17.3 (5.1)	16.5 (6.8)	0.7887
Discharge	PANSS Disorganised Factor – Mean (SD)	8.7 (2.7)	8.9 (2.0)	0.6825	8.9 (2.4)	7.0 (2.6)	0.1798
	PANSS Excited Factor – Mean (SD)	8.0 (2.9)	8.0 (2.7)	0.9033	8.0 (2.7)	8.5 (4.4)	0.9695
	PANSS Depressed Factor – Mean (SD)	5.5 (1.9)	5.4 (2.3)	0.7252	5.5 (2.1)	5.0 (1.8)	0.7113
	mSAS – mean (SD)	0.2 (0.4)	0.3 (0.3)	0.3156	2.4 (3.4)	3.0 (6.0)	0.4978
	AIMS TS – mean (SD)	1.1 (2.2)	0.1 (0.3)	<0.05	0.6 (1.6)	1.8 (3.5)	0.7304
	Tardive dyskinesia	5 (12.5%)	0 (0.0%)	0.0562	4 (6.35%)	1 (25.0%)	0.1687
	PSP	54.9 (13.2)	53.1 (10.7)	0.6181	53.6 (11.8)	62.8 (18.2)	0.2963

We noted a correlation between acute toxoplasmosis infection (based on the level of IgM class antibodies) and the course of schizophrenic psychosis. The Toxo-IgM (+) patients had higher values of PANSS positive factor and focal symptoms subscales upon admission compared to Toxo-IgM (-) patients with a significant correlation of symptoms and antibody titres (Table 3). Additionally, at discharge the PANSS excitement factor increased together with an increase in the Toxo-IgM titre for both Toxo-IgM (+) ($R = 0.41$; $p < 0.01$) and Toxo-IgM (-) patients ($R = 0.52$; $p < 0.01$). No statistically significant differences were observed in the scope of other subscales of the PANSS scale including factors of PANSS-FCTcr on admission, at discharge or comparing the admission period with the discharge period.

Toxo-IgM (+) patients had lower quality of life as measured by the PSP scale when admitted compared to Toxo-IgM (-)

patients, and these results correlated with the antibody concentration (Table 2 and 3). There were no differences in this scale at discharge and there were no differences in the distribution of the PSP subscales.

Toxo-IgM (+) patients had a longer duration of illness, involuntary hospitalisation, lower Toxo-IgM (-) patients (Table 2). There were no patients taking drugs for the *T. gondii* infection in the analysed period.

The main limitation of our study is the small number of patients with positive (6%) or doubtful (3%) Toxo-IgM index. The post-hoc analysis comparing Toxo-IgM (+) groups showed only 43.7% statistical power of the IgM calculations. To reach statistical significance threshold (Alpha = 0.05 Beta = 0.2 and Power = 0.8), future studies should involve a sample size of at least 188 (94 females + 94 males).

Table 3. Correlations of clinical scales with antibodies level

		IgG level		IgM level	
		Spearman	P-value	Spearman	P-value
On admission	Total PANSS	0.07	0.65	0.19	0.24
	Focal PANSS	- 0.04	0.82	0.32	0.04
	Positive factor	0.09	0.59	0.40	0.01
	Negative factor	- 0.18	0.27	0.06	0.73
	Disorganized/concrete factor	0.24	0.14	0.09	0.60
	Excited factor	0.21	0.19	0.21	0.19
	Depressed factor	- 0.14	0.38	- 0.25	0.12
	mSAS	- 0.01	0.96	- 0.11	0.50
	AIMS total severity	- 0.12	0.45	0.02	0.90
	AIMS global severity	- 0.14	0.39	-0.02	0.91
	PSP	0.03	0.86	- 0.37	0.02
On discharge	Total PANSS	0.02	0.90	0.21	0.20
	Focal PANSS	0.03	0.84	0.21	0.19
	Positive factor	0.09	0.59	0.19	0.23
	Negative factor	- 0.06	0.70	0.09	0.56
	Disorganized/concrete factor	0.13	0.42	0.09	0.57
	Excited factor	0.02	0.90	0.40	0.01
	Depressed factor	- 0.33	0.04	- 0.11	0.50
	mSAS	0.03	0.87	0.15	0.37
	AIMS total severity	- 0.11	0.50	0.02	0.92
	AIMS global severity	- 0.14	0.39	- 0.02	0.91
	PSP	0.13	0.43	- 0.20	0.22

For Toxo-Ig, an independent variable, the stepwise multivariate binary logistic regression model was built using the significant results from the univariate analysis (Table 2). After eliminating statistically insignificant results, finally 4 variables were included in the multivariate model (Somers' D = 0.69; Kolmogorov–Smirnov statistic = 0.55; $p = 0.0001$). The analysis revealed that the factors reducing the likelihood of Toxo-IgG (+) were: < 40 years of age (OR 0.54, 95% CI 0.29-1.00; $p = 0.04$), no obesity (OR 0.36, 95% CI 0.15-0.89; $p = 0.03$) and male gender (OR 0.35, 95% CI 0.16-0.76; $p = 0.008$). The only factor significantly associated with an increased likelihood of Toxo-IgG (+) was lower education (primary or vocational school) OR 2.41, 95% CI 1.21-4.79; $p = 0.01$ (Figure 1).

Discussion

We present the toxoplasmosis immune status in a case series study of patients acutely hospitalised due to an exacerbation of schizophrenia. Many significant differences were observed, both in the demographic characteristics and in the scales assessing the severity of disease symptoms. Seropositive patients significantly differ from the seronegative patients mainly in terms of sex, age, BMI and education level. Acute toxoplasmosis (assessed as IgM (+)) seems to be significantly associated with higher rates of positive symptoms (as measured by the PANSS scale). It is important to note there was limited sample size of IgM-positive subjects in our study.

The occurrence of latent toxoplasmosis is estimated at up to 30% of the world's population. Various studies have shown that it can influence the host's behaviour by influencing neurotransmitters [18]. Many studies have suggested the influence of *T. gondii* infection on the development of schizophrenia [18-20].

The exact molecular and cellular mechanisms underpinning the association between toxoplasmosis and schizophrenia remain poorly understood [21-22]. Various mechanisms explaining the influence of infection on human behaviour have been proposed, e.g. alterations in neurotransmitter release, cyst location, and neuroinflammation [23-24]. The causality of the link between *T. gondii* and schizophrenia is also unclear. This infection may spread by eating undercooked meat or unwashed fruits and vegetables containing protozoan oocysts [25-26]. This may be a result of negligence accompanying the predominant negative and disorganisation symptoms of schizophrenia, so in such case a *T. gondii* infection would be secondary to schizophrenia. An indirect proof of this hypothesis may be found in a meta-analysis which demonstrated a significant increase in prevalence of positive *T. gondii* IgM antibodies in patients with acute psychosis compared with controls, while the observed association was stronger for patients with chronic schizophrenia than those with a first episode of psychosis [27]. Similarly, increased *T. gondii* seropositivity was also observed in patients with treatment-resistant schizophrenia [28].

However, the relationship between *T. gondii* infection and schizophrenia seems even more complicated. A large survey

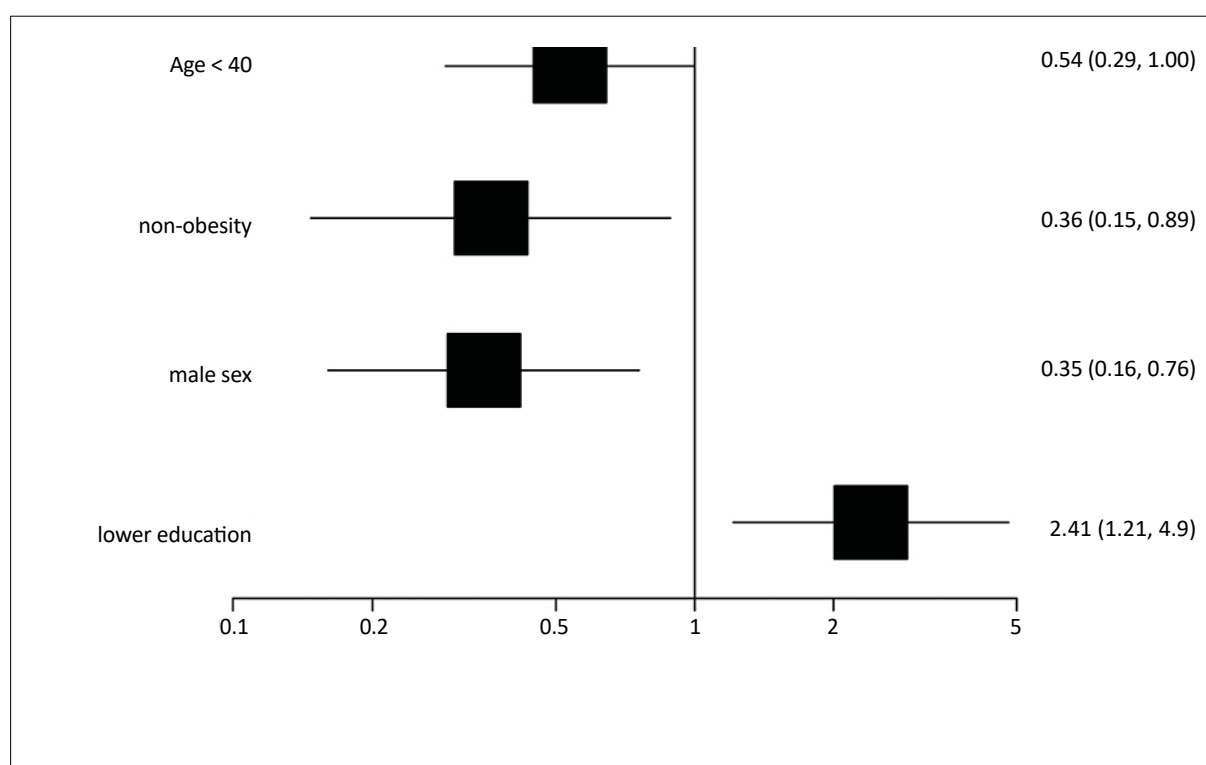


Figure 1. Multivariate logistic regression

of military members showed significant associations between increased levels of scaled *T. gondii* IgG antibodies and schizophrenia (measured prior to and after diagnosis) [29].

Few data exist concerning the clinical correlates of toxoplasmosis in persons with schizophrenia [5, 18, 30-31]. Patients with a latent *T. gondii* infection were proven to have 15 times higher probability of experiencing continuous course of disease than *Toxoplasma*-free subjects [32]. *T. gondii* seropositive patients with schizophrenia in general were found to have a worse course of schizophrenia and more severe positive symptoms [33]. The same study showed that higher Positive and Negative Syndrome Scale (PANSS) scores were associated with lower titres of anti-Toxoplasma antibodies suggesting that psychopathology may deteriorate with duration of that parasitic infection. Another study suggested that seropositive subjects present poor impulse control, personality aberrations or neurocognitive impairment, as well as a higher risk of suicide [34]. There are also studies suggesting there is no correlation between latent toxoplasmosis and schizophrenia symptomatology at all [23, 35].

According to the authors of a 2020 meta-analysis, *T. gondii* infection has a negligible impact on the intensity of positive and total symptoms of schizophrenia in people who are in the early stages of that disease [18]. Although further research is still required, this is consistent with the theory that *T. gondii* infection is directly associated with schizophrenia. [18].

Our results show a correlation between the course and symptomatology of schizophrenia and acute or latent infection with *Toxoplasma gondii*. Higher PANSS positive and excitement factor scores in patients with positive anti-Toxoplasma IgM class antibodies may point towards a sometimes postulated CNS metabolism change induced by *T. gondii* and leading to dopamine overproduction with increased dopamine concentration in the mesolimbic pathway being associated with positive symptoms of schizophrenia [36]. Higher severity of these symptoms may, by disorganising behaviour, also lead to lower quality of life in patients with higher Toxo-IgM antibodies that was observed in this study. Perhaps latent toxoplasmosis in the course of schizophrenia may lead to a more severe positive psychopathology and perhaps less favourable course of schizophrenia. Holub et al found that *T. gondii*-infected patients with schizophrenia scored higher in the positive subscale of the PANSS and had higher scores in Total PANSS [33]. The authors also found that infected patients remained in the hospital for about 33 days longer during their most recent admission than the uninfected ones [33].

The age effect seems to be interesting as well. Some studies suggest that the percentage of *T. gondii* seropositive people increases with age [37]. In our study, we found that age below 40 was reducing the likelihood of Toxo-IgG (+). On the contrary, according to Kezai et al, *T. gondii* seroprevalence was found to significantly increase with age in controls, but this association was not seen in patients with schizophrenia [30]. That study sample included a high percentage of seropositive

subjects who were under the age of 38 [30]. Based on this, the authors claimed that *T. gondii* infection may contribute to the development of schizophrenia. In a recent case-control study, Ademe et al observed more *T. gondii* infections among schizophrenia patients who were male and > 35 years of age [38]. This contrasts with our results, which suggest that men are less likely to be seropositive. Single studies have examined the role of education level on *T. gondii* serostatus, finding no statistically significant associations [37].

The data from a German study were significant for cross-sectional studies in the general population [39]. Seroprevalence increased from 20% in the 18-29 age group to 77% in the 70-79 age group, according to the authors' analysis of a representative sample of 6,663 individuals. Younger males and older females had higher seroprevalences, researchers found. Being male, overweight or obese was positively correlated with seropositivity. Having a high socioeconomic status was a protective factor for *T. gondii* infection [39].

Our results highlighted that compared to Toxo-IgG (-) patients, Toxo-IgG (+) patients received mean lower doses of antipsychotic drugs and were more frequently treated with monotherapy. In another study it was also found that patients with a latent toxoplasmosis required lower doses of antipsychotics than seronegative patients (at least in terms of assessed hospitalisation), yet involuntary movements and tardive dyskinesia were more frequent, which is another argument for the possible influence of *T. gondii* infection on neurotransmission [40]. Lower doses of CPZE may also indicate a self-restricting character of psychosis in Toxo-IgM (+) patients due to alleviation of acute toxoplasmosis.

On the other hand, patients with positive Toxo-IgG antibodies have more prevalent parameters that may indirectly indicate lower self-care: more frequent obesity, higher glucose levels, lower education and less frequent psychotherapy. This may suggest that those initially less aware of or less willing to apply healthy behaviours are predisposed to toxoplasmosis infection (for instance by eating undercooked meat, unwashed vegetables), which could partly explain the high prevalence of toxoplasmosis in patients with schizophrenia where neglect is often a result of negative symptoms of the disease [41]. This argument is also supported by a higher mean age and longer duration of the disease in patients with a history of toxoplasmosis, and thus a longer period of risky behaviour [18]. The connection between Toxo-IgG (+) and the female gender remains unclear, requiring more detailed analyses.

Potential explanation of these contradictory hypotheses could be that schizophrenia predisposes to toxoplasmosis, which in turn has its role in a relapse of psychosis by altering neurophysiological mechanisms in CNS. Another view may be that increased IgM antibodies are not a marker of recent infection in these patients, but rather a sign of reinfection of latent toxoplasmosis associated with a relapse of schizophrenia [5].

Future studies on both Toxo-IgM (+) and Toxo-IgG (+) subjects with schizophrenia should include neuroimaging, particularly assessing dopaminergic neurotransmission, neuroinflammatory changes or lesions within the CNS. There is some evidence that antipsychotic drugs may inhibit tachyzoite replication in vitro and reduce *T. gondii* antibody titres, which could be investigated closely [22].

Presumed differences between patients with and without toxoplasmosis could suggest a different basis for similar schizophrenia symptoms, which would entail further steps such as assessing the validity of toxoplasmosis screening in the context of expected different treatment efficacy and a possible consideration of including anti-protozoan drugs in the treatment of patients with schizophrenia.

Several of the limitations associated with the descriptive nature of our study must be addressed. Based on our results, we were not able to find out how *T. gondii* infection could be related to schizophrenia, particularly because there we did not have a comparison group. The presented study groups were given to offer a possible explanation for how *T. gondii* infection may play a part in the pathogenesis of schizophrenia. However, the main limitation in this regard was inability to make many because there was no reference group.

Our study was conducted at a single site during a single patient hospitalisation. This makes it impossible to draw conclusions about cause-effect relationships. The number of patients recruited for the study was too small, especially for Toxo IgM (+) patients. As power analysis demonstrated, the sample size too small. In addition, there is the lack of verification of enzyme-linked immunosorbent assay (ELISA) tests by polymerase chain reaction (PCR).

One of the most important results in logistic regression turned out to be age and gender, but it should be noted that the groups of women and men differed statistically in age and the dose of the antipsychotic drug received (Table 1). Therefore, these results should be interpreted with caution.

In addition to the discussion of the relationship between *T. gondii* infection and schizophrenia, there is a need for additional evidence beyond the correlation of these two factors, especially using the Bradford Hill criteria for assessing causal relationships in observational data [11].

Overall, the study findings may indicate a particular role for *T. gondii* infection in schizophrenia patients. We believe that this study offers a significant starting point for additional research. Our findings imply that monitoring *T. gondii* infection in schizophrenia patients may have clinical implications for issues like symptom severity and antipsychotic medication doses.

Conclusions

Seropositive toxoplasma patients were mainly older females with obesity and lower level of education. Acute toxoplasmosis seems to be significantly associated with higher rates of positive symptoms of schizophrenia. Patients with Toxo IgG (+) received lower mean doses of antipsychotics and were more frequently treated with monotherapy. However, the small number of patients and descriptive nature of this study suggests that its results should be interpreted with caution.

Acknowledgements

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Data Availability Statement

The data that support the findings of this study are not openly available (due to containing sensitive human data) and are available from the corresponding author upon reasonable request.

Ethical considerations

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Funding

This study was supported by grant MN – 01-0246/08/222 funded by the Polish Ministry of Science and Higher Education.

Conflicts of interests

None.

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The prevalence of obesity diagnosed by different diagnostic criteria in Polish social-media users

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Abstract

Background: In 2020, Polish experts recommended the use of the guidelines of the American Association of Clinical Endocrinologist and the American College of Endocrinology (AACE/ACE) in the diagnosis of obesity instead of the 1998 WHO criteria. Thus, obesity can be also diagnosed in patients with BMI 25.0-29.9 if there are complications related to the excess body weight including infertility and hypogonadism, asthma, sleep apnea, gastroesophageal reflux disease, stress urinary incontinence, osteoarthritis and depression. **Material and methods:** The aim of the study was to assess the prevalence of obesity diagnosed according to the new criteria in the group of Polish social media users. **Results:** Among the respondents, 9.7% had a BMI below 18.5 kg/m², 63.9% declared normal BMI, and 18.8% had BMI 25.0-29.9 kg/m². The value of BMI ≥ 30 kg/m², equivalent to the diagnosis of obesity according to WHO, was found in 7.6% of the respondents. For comparison, the prevalence of obesity according to the AACE/ACE criteria was more than twice as high (17.2%, $p < 0.05$). **Conclusions:** Concluding, according to the new criteria, more respondents were diagnosed with obesity compared to WHO criteria. The results of the study indicate that the application of the new diagnostic criteria may enable earlier diagnosis and thus earlier treatment of obesity.

Keywords: obesity · body mass index · AACE/ACE diagnostic criteria for obesity · complications of obesity

Citation

Samek G, Szulc A, Dardzińska JA. The prevalence of obesity diagnosed by different diagnostic criteria in Polish social-media users. Eur J Transl Clin Med. 2023;6(1):25-30.

DOI: [10.31373/ejtcmed/162572](https://doi.org/10.31373/ejtcmed/162572)

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Available online: www.ejtcmed.gumed.edu.pl

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Introduction

In accordance with the World Health Organization (WHO), obesity is a chronic disease with a complex etiology and serious consequences [1]. This is undoubtedly demonstrated during the current SARS-CoV-2 pandemic as obese people have a more severe course and a longer duration of coronavirus infection due to immunodeficiency secondary to obesity. Other common complications of obesity, e.g. type 2 diabetes, left ventricular hypertrophy, hypertension and heart failure and prothrombotic state also contributed to a vastly increased risk of hospitalization or even death from COVID-19 in this group of patients [2-6]. Unfortunately, as if that were not enough, obesity is also associated with a psychological burden, such as lowered self-esteem, depressed mood, decreased quality of life and emotional stress, all of which can significantly interfere with treatment [7-8]. On the other hand, exposure to chronic stress may promote the development of obesity. One of the mechanisms involved may be the compensation of negative emotions with food [2, 8]. Increasingly, overeating is a way to reduce anxiety and improve mood [9-10]. Obesity is considered serious public health problem and ranks fifth as the world's leading cause of death. The WHO predicts that by 2030 as many as 30% of deaths around the world will be initiated by diseases of civilization. This process can only be stopped by properly identifying diseases, addressing related risk factors and a policy of behavioral involvement. Therefore, early diagnosis of obesity is crucial for effective multimodal treatment [11].

According to the 1998 WHO criteria, the body mass index (BMI) is the most widely used tool in the diagnosis of obesity [1]. However, this tool has many disadvantages [2, 8, 12]. Most importantly, the BMI does not address the distribution of body fat, while it is known that a relevant percentage of adults who are overweight but have BMI < 30 kg/m² experience complications from excess visceral fat [13-15]. According to the research and data of the Statistics Poland (Główny Urząd Statystyczny), in 2020 BMI ≥ 30 kg/m² concerned 18.5% of the Polish society [16]. These data are overwhelming, but it should be noted that BMI 25.0-29.9 was found in a much larger group of nearly 19 million Polish citizens [16]. Unfortunately, patients with such BMI are considered overweight and not obese, thus may be overlooked by general practitioners.

Therefore, attention should be paid to the new guidelines for the diagnosis of obesity presented by the American Association of Clinical Endocrinologists and the American College of Endocrinology (AACE/ACE) in 2016 [17] and recommended for Polish patients in 2020 by the Polish Association for the Study of Obesity (PTBO) [2]. These guidelines are not based solely on BMI, but also take into account the presence of diseases and clinical conditions that may be metabolic consequences of excess body fat, such as

metabolic syndrome and insulin resistance, type 2 diabetes, cardiovascular disease, arterial hypertension, dyslipidemia, non-alcoholic fatty liver disease, female infertility, polycystic ovary syndrome, male hypogonadism, asthma, sleep apnea syndrome, hypoventilation syndrome, gastroesophageal reflux disease, osteoarthritis, stress urinary incontinence and depression [2, 17]. Obesity can be diagnosed in people with a BMI in the range of 25.0-29.9 if they also have one of the aforementioned complications [2, 17]. Thus, the new guidelines allow for an earlier diagnosis of obesity and consequently a more rapid implementation of treatment.

The main aim of our work was to rate the prevalence of obesity diagnosed according to these new criteria and to compare the results with the WHO criteria. Due to the limitations associated with the pandemic, social media users were selected for this pilot study.

Material and methods

This was an online retrospective observational survey conducted using social media in February and March 2022, during one of the waves of the SARS-CoV-2 pandemic in Poland and the outbreak of the war in Ukraine. Participation in the study was unsolicited and did not involve any financial benefits. The only inclusion criteria were age over 18 and consent to participate in the examination.

In the survey we asked about the respondents' place of residence, education, socioeconomic status, current weight, height, waist circumference and changes in body weight over the past two years, medication used and the presence of complications of obesity that are listed above. The survey link was shared via various social media and applications (Facebook, Instagram and WhatsApp), to ensure that a diverse group of participants could be reached.

BMI was calculated based on the height and weight provided by the respondents. Based on their BMI, the respondents were classified into following four groups: underweight (BMI < 18.5 kg/m²), normal weight (BMI 18.5-24.99 kg/m²), overweight (BMI 25-29.99 kg/m²) and obese (BMI ≥ 30.0 kg/m²).

Obesity was diagnosed according to one of the following criteria:

1. if the BMI ≥ 30.0 kg/m² (WHO) [1],
2. if the declared waist circumference were at ≥ 80 cm in women and ≥ 94 cm in men (International Diabetes Federation) [18],
3. if BMI in the range of 25.0-29.9 kg/m² and the respondent declared having at least one of the complications of obesity listed in the AACE/ACE guidelines [2, 17].

All statistical calculations were done by using the Statistica software (version 13.3, StatSoft, USA). Quantitative

variables were presented as the mean and standard deviation (SD) and qualitative variables were presented in terms of numbers or percentages. The significance of differences between the two groups (model of unrelated variables) was tested by Student's t-test or the U Mann-Whitney test. Chi-square tests of independence were used for qualitative variables. The significance level of $p = 0.05$ was assumed in all calculations.

Results

580 people aged 18 to 80 participated in our study. Most of them (65%) were from cities with > 100,000 inhabitants and declared an average financial status. As many as 81% of the respondents were women. Men were significantly older than women (mean age 30.0 ± 10.0 years vs. 27.1 ± 9.7 years, $p < 0.05$). Smoking cigarettes (traditional and electronic) was declared by 22% of the respondents. The respondents' BMI ranged from 16.0 to 40.0 kg/m^2 . The classification of patients with different categories of BMI is presented in Figure 1. BMI was higher in men than in women: $25.3 \pm 3.9 \text{ kg/m}^2$ vs. $22.8 \pm 4.3 \text{ kg/m}^2$ ($p < 0.05$).

The prevalence of obesity in the studied population differed depending on which diagnostic criteria were used. As presented in Figure 1, according to the WHO criteria obesity was found in 7.6% of the respondents. However, according to ACEE/ACE criteria, its prevalence was more than twice as high (17.2%, $p < 0.05$). Taking into account the declared waist circumference, abdominal obesity was found in 25.2% of the respondents (Figure 2). The prevalence of obesity diagnosed according to ACEE/ACE criteria was higher in men than in women (data presented in Table 1). During the pandemic, as many as 43% of respondents experienced weight gain ($6.2 \pm 4.2 \text{ kg}$). The most common obesity-related disease or complications are presented in Table 2. The relationship between age and BMI among women and men and in the subgroup of respondents declaring a diagnosis of depression and without such a diagnosis is presented in Figures 3 and 4.

Discussion

To our knowledge, this is the very first Polish research to diagnose obesity according to the ACEE/ACE guidelines and it was found that the prevalence according to the ACEE/ACE criteria was more than twice as high as that diagnosed traditionally with WHO cut-off points for BMI. Such results may indicate the limitation of the only use of simple anthropometric measurements in such a complex diagnosis as obesity and the need to screen a patient with a BMI in the range

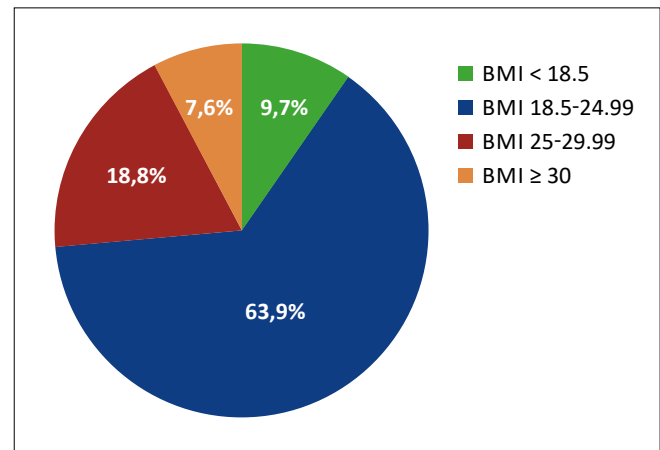


Figure 1. Categories of BMI in all respondents (n = 580)

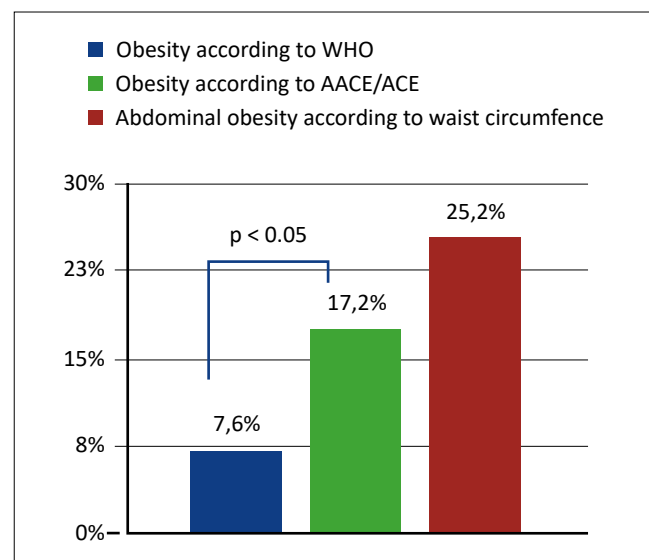


Figure 2. The prevalence of obesity diagnosed by different diagnostic criteria (n = 580)

of 25-29.9 for obesity-related complications. On the other hand, our study showed that waist circumference measurement must be included in the comprehensive patient assessment, particularly because the number of people with excess weight is seriously increasing. As many as 43% of our respondents declared that they gained an average of 6 kg over the last two years. This is similar to the data obtained in the Ipsos Covid 365+ study in a representative sample of

Table 1. Prevalence of obesity according to different criteria in men and women

Criteria	Men n = 105	Women n = 475	Chi-square P value
WHO	11.4%	7.2%	0.1
AAEE/ACE	24.8%	15.6%	0.02
IDF (abdominal obesity)	31.4%	23.8%	0.1

Table 2. The prevalence of obesity-related disease or complication among respondents

Obesity-related disease or complication	Respondents with obesity according to ACEE/ACE (n = 100)	Respondents with obesity according to WHO (n = 44)	All (n = 580)
Insulin resistance or prediabetes	26.0%	31.8%	7.4%
Diabetes type 2	2.0%	4.5%	0.3%
Lipid disorders	8.0%	2.3%	2.7%
Arterial hypertension	26.0%	29.5%	5.9%
Cardiovascular disease	3.0%	2.3%	0.8%
Non-alcoholic fatty liver disease	5.0%	4.0%	2.1%
Polycystic ovary syndrome	7.0%	4.5%	5.1%
Female infertility and male hypogonadism	2.0%	2.3%	1.5%
Asthma	12.0%	11.4%	6.4%
Sleep apnea and hypoventilation syndrome	2.0%	2.3%	0.8%
Gastroesophageal reflux	21.0%	18.2%	9.8%
Stress urinary incontinence	10.0%	11.4%	2.4%
Osteoarthritis	3.0%	6.8%	1.9%
Depression	38.0%	29.5%	18.6%
At least one of the following	87.0%	70.5%	40.2%

1,004 people [2, 8, 10]. Our results are also in line with the data from the WOBASZ study for the youngest group (20-34.9 years) [19].

The selection of the group for our pilot study was dictated by the epidemiological situation. Nevertheless, the PTBO guidelines developed during the pandemic strongly recom-

mended the use of telemedicine techniques to reach as many patients at risk as possible. Completing an online survey could raise health awareness in people who have never had direct contact with health services [2]. Telemedicine has been crucial during the pandemic and continues to be increasingly popular due to its convenience. Our results indirectly indicate that telemedicine can be helpful in the selection of young people at high risk of developing chronic diseases. On the other hand, through social media, health education adapted to young can and should be conducted. Experts also emphasize the importance of cooperation with a dietitian in the prevention of obesity complications. Unfortunately, lack of reimbursement for such services makes such cooperation impossible within the current the Polish healthcare system. On the other hand, the frequent coexistence of excess weight with psychological consequences indicates the need to extend the therapeutic team to mental health specialists. Obesity is associated with a wide range of mental effects,

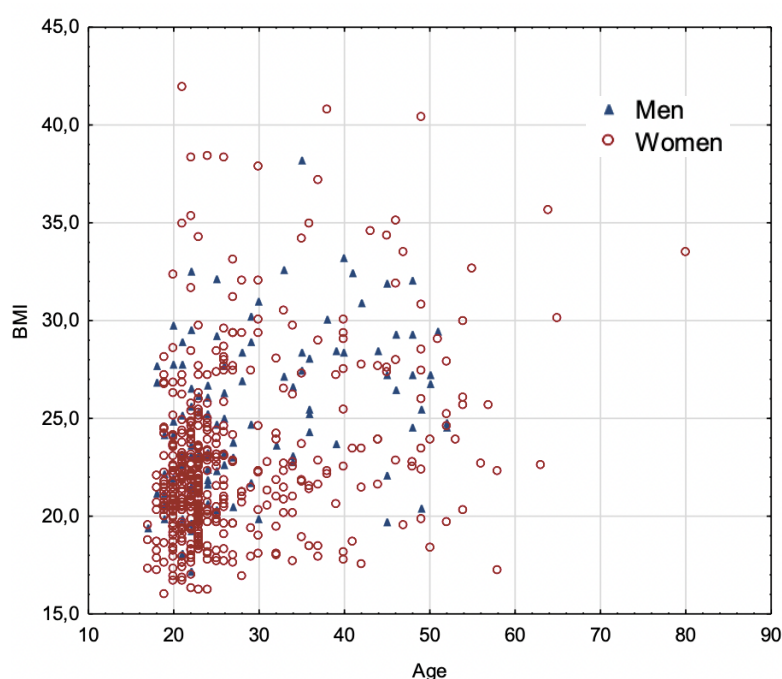


Figure 3. The relationship between age and BMI among women and men

including depressed mood, low self-esteem, impaired body image, stress, eating disorders and poor quality of life. Comprehensive treatment, in addition to dietary recommendations and exercise, should include cognitive behavioral therapy with lifestyle interventions [7, 20]. The development of telemedicine may eliminate at least some of the barriers in access to specialists [21-22].

Our results may confirm a link between depression and being overweight described by others [7, 20]. This is a very worrying phenomenon because the majority of people suffering from depression are young [23]. The reasons for this are complex and it cannot be ruled out that broad access to new technologies plays a role here [20, 24]. The limitation of our pilot study was the small group of respondents and the lack of direct contact with the subjects. For these reasons, our results may be underestimated. The overrepresentation of women in the study group is another limitation of our work. The form of online contact with study participants made it impossible for us to measure the respondents' weight, height and waist circumference or to perform the oral glucose tolerance test and analyze its results. Therefore, we asked also about the diagnosis of insulin resistance, as this term is more understandable for internet respondents. Taking into account that type 2 diabetes and prediabetes are underdiagnosed, this difference may not be significant. Studies on a larger representative sample are needed to confirm the results of our small pilot study.

Conclusions

The results of our study may indicate the problem of underestimating the risk of complications in young people with

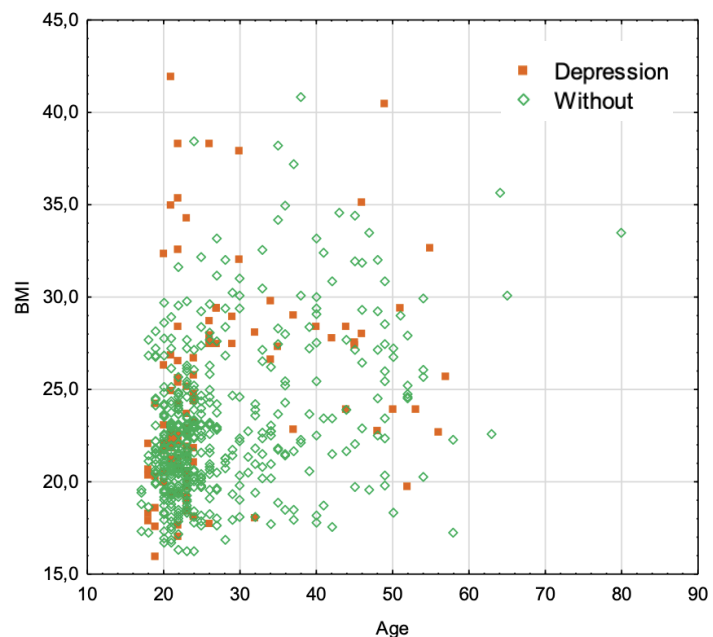


Figure 4. The relationship between age and BMI in respondents declaring a diagnosis of depression and without such a diagnosis

a BMI in the range of 25-29.9. Diagnosing obesity according to the ACEE/ACE guidelines may enable earlier diagnosis and thus earlier treatment of obesity.

Funding

None.

Conflicts of interest




There is no conflict of interest in this project.

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Analysis of diabetes-related complications and the cost of diabetes in Poland. The methodology and the research sample used in *Economedica DM-PL* Survey

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Abstract

Background: Knowledge about diabetes mellitus (DM)-related complications, the direct and indirect costs incurred by patients with DM is still insufficient in Poland. *Economedica DM-PL* (*Economedica*) survey was a cross-sectional observational study that aimed to assess these issues. **Material and methods:** The survey was carried out in 2016 on a representative sample of 2032 diabetic patients recruited by 195 general practitioners (GP) and 48 diabetologists (D) randomly selected from the national registry of physicians. Each GP and D recruited 8 to 10 patients with DM according to the specified protocol. The study included 1938 medical questionnaires (completed by the GPs and Ds), 1938 questionnaires about costs (completed by the patients) and 1532 treatment adherence questionnaires. **Results:** The *Economedica* survey provided new insights into DM epidemiological and cost data. 70% of patients with type-1 DM were diagnosed with the disease before the age of 40. For 76% patients with type-2 DM, it was diagnosed after the age of 50. The average monthly out-of-pocket medical costs for DM patients in 2016 reached 66.2 EUR, while their non-medical costs were 36.8 EUR. **Conclusions:** The methodology applied in our study proved useful for providing new dimensions of DM epidemiological and cost data in Poland.

Keywords: diabetes mellitus · prevalence · cost of illness · research design



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Citation

Bogusławski S, Wojciechowska A, Smaga A, Strojek K, Wróbel K, Wojtyniak B, Juszczak G, Czech M, Zdrojewski T. Analysis of diabetes-related complications and the cost of diabetes in Poland. The methodology and the research sample used in *Economedica DM-PL* Survey. Eur J Transl Clin Med. 2023;6(1):31-40.

DOI: [10.31373/ejtc/162189](https://doi.org/10.31373/ejtc/162189)

Abbreviations

CATI – computer assisted telephone interview

D – diabetologist

DM – diabetes mellitus

FFS – fee for service

GP – general practitioner

HDM – Health Data Management Ltd.

NFZ – Narodowy Fundusz Zdrowia (National Health Fund)

NIZP-PZH – Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny – Państwowy Instytut Badawczy (National Institute of Public Health – National Institute of Hygiene – National Research Institute)

PESEL – Powszechny Elektroniczny System Ewidencji Ludności (national ID number)

through subscription and insurance companies) is quite fragmented. As a result, the number of DM patients who receive care and treatment in this manner is unknown.

The *Economedica* study was designed to obtain objective data from a representative sample of DM patients treated by GPs and diabetologists (Ds) in Poland about the prevalence of DM complications and various types of DM-related costs with a relatively simple and easily reproducible methodology. The goals of the *Economedica* survey were to evaluate:

- the prevalence of the main DM related complications in a representative sample of adult patients with DM;
- the direct medical and non-medical costs incurred by patients with DM;
- the indirect costs of treatment of DM and its complications;
- the level of adherence to treatment among patients with DM.

The aim of this paper was to describe the methodology and some results of the *Economedica* study.

Introduction

The World Health Organisation forecasts a steady increase in the rates of obesity and diabetes mellitus (DM) [1]. It is estimated that, globally, the number of people suffering from DM will increase from 382 million in 2013 to 592 million in 2035 [2]. In 2004 the number of patients with DM in Poland was projected to reach 2.5 million by 2030 [3], but according to the available data, this number was already at 2.68 million in 2014 [4], with DM affecting 8.4% of the general adult population, similarly to the rate observed in other European populations [5]. Clinical data shows that DM, if incorrectly treated, leads to severe complications affecting most bodily organs. Therefore, DM in middle and high income countries constitutes a heavy burden on the finances of public and private payers. At the same time, the course of DM can be positively modified by early diagnosis and through the adoption of appropriate drug therapies and changes in lifestyle.

The existing data on the costs of DM in Poland, which are based on the diagnoses and the services provided to patients by National Health Fund (Narodowy Fundusz Zdrowia – NFZ), the only public payer, is incomplete [4]. The incomplete data results from widespread upcoding in the system of reported diagnoses and linking the services to diagnoses that are most cost-intensive, as well as from the gaps in the data reported by general practitioners (GPs). At the same time, the data obtained from the rapidly growing non-public sector (buying services on a fee for service basis, FFS) and

Material and methods

Random sampling of general practitioners and diabetologists

The survey was designed as an observational study on a representative sample of DM patients treated by randomly selected GPs and Ds in Poland. The sample of GPs and Ds was drawn from the database of Health Data Management (HDM), a continuously updated address list of practicing physicians in Poland. We planned sample sizes of 200 GPs and 50 Ds working in outpatient clinics. According to the HDM data, at the time of the study there were 20944 practicing GPs and 955 practicing Ds in Poland [6]. The following quoting mechanism was proposed. In the case of the GP sample, geographical strata were created on the basis of Poland's 16 provinces (województwa) and three types of locations: village/town with a population < 20000; town/city with a population between 20001 and 100000; and city with a population > 100000. For the D sample, two location categories were adopted: < 100000 and > 100000 population. In case of refusals, the remaining physicians were recruited using quota sampling. Ultimately, 243 physicians took part in the study: 195 GPs and 48 Ds.

Random sampling of patients with DM

Each participating GP recruited 8 patients with DM (including one patient with type-1 DM) to the study, completed medical questionnaires about them and asked patients to complete cost questionnaires. Each D recruited 10 patients (including 6 with type-1 DM). The inclusion criteria were: patient > 18 years with a prescription for at least one hypoglycemic medication during the visit. Among those patients who met the inclusion criteria, the GPs qualified the first patient with type-1 DM and every third patient with type-2 DM, while the Ds qualified every second patient with type-1 DM and every third patient with type-2 DM. Additionally, the GPs and Ds prepared a register of all the patients they consulted over a period of two weeks, including their age, sex and diagnosis. The diagnosis of type-1 or 2 DM was based on the patients' medical records. The study was carried out between March and July 2016.

Study design

DESIGN OF THE PROJECT

Week 1-2:

General practitioners and diabetologists:

1. Registry of all patients consulted in outpatient clinic (age, sex and diagnosis)
2. Random recruitment of 8 to 10 adult patients with diabetes and filling out:
 - a. the medical questionnaire by the physicians;
 - b. the costs questionnaire by patients.

Week 3-8:

Computer Assisted Telephone Interview to assess adherence to treatment

- random recruitment (in the order described above) of patients with DM during routine visits: 8 in the case of the GP's and 10 in the case of Ds. For all the recruited patients, the physicians completed a medical questionnaire, whereas the patients completed questionnaires about costs [including medical and non-medical costs incurred by the patients as well as indirect costs of DM, e.g. absenteeism (absence from work) and presenteeism (working while sick, resulting in reduced work effectiveness)] [7-8];
- a computer assisted telephone interview (CATI) carried out during the subsequent 6 weeks (weeks 3 to 8), to assess whether the patients adhered to the treatment they were prescribed after their consultation with the GP or D.

The medical questionnaire completed by the physicians included the following information:

- age, sex, type of DM and its treatment, BMI, blood pressure;
- presence of confirmed DM-related complications, e.g. ischemic heart disease, chronic heart failure, stroke, peripheral vascular disease, diabetic foot syndrome, amputation associated with peripheral vascular disease/diabetic foot syndrome, diabetic nephrectomy, kidney transplant, diabetic retinopathy, diabetic maculopathy, secondary glaucoma, diabetic neuropathy and depression/depression treatment;
- occurrence of hypoglycemic episodes and a description of the episodes;
- presence of risk factors: hypercholesterolemia, hypertriglyceridemia, arterial hypertension, tobacco smoking, lack of physical activity, a diet high in saturated fats;
- last HbA1c and lipid profile laboratory results.

The costs questionnaire, which was completed anonymously by the patients during their visit with the GP or D included the following information:

- the medical costs of DM and/or its complication incurred in the last 4 weeks;
- the non-medical costs of DM and/or its complication incurred in the last 4 weeks (e.g. food, cosmetics, transport, care, etc.);
- the level of income lost by the patient's family members in connection with DM patient care and its complications;
- absenteeism or presenteeism due to DM and/or its complications, the use of medical services (visits to specialists in the last 4 weeks) and/or hospital treatment;
- data concerning the glycaemia measurement methods used by the patient (based on the patient's declaration);
- education and per capita household income.

The Computer-Assisted Telephone Interviews were carried out with patients on average 30 days after the visit to the physician (between weeks 3 and 8 of the study) and focused on the results of glycaemia measurements, the details of the use of antidiabetic drugs in relation to the physician's instructions and the reasons for potential non-adherence,

Figure 1. Design of the Economedica DM-PI Survey

The *Economedica* survey involved:

- registration of all patients (including data about the age, sex, diagnosis) consulted by the GPs and Ds in their clinical practice;

Table 1. Age and gender structure of the DM patients' sample (raw data)

Sample structure	Type 1 diabetes		Type 2 diabetes		Total	
	N	% of the sample	N	% of the sample	N	% of the sample
Age groups						
20-29	87	21%	6	0,4%	93	5%
30-39	113	27%	34	2%	147	8%
40-49	92	22%	111	7%	203	10%
50-59	63	15%	287	19%	348	18%
60-69	53	13%	631	42%	684	35%
70-79	13	3%	350	23%	363	19%
80 or older	1	0.2%	99	7%	100	5%
Sex						
Female	217	52%	789	52%	1006	52%
Male	203	48%	729	48%	932	48%

N – number of individual patients

Table 1a. Age and gender structure of the DM patients' population after projection (weighted data)

Sample structure	Type 1 diabetes	Type 2 diabetes	Total
	% of the sample	% of the sample	% of the sample
Age groups			
20-29	10%	0,3%	1%
30-39	14%	2%	3%
40-49	12%	5%	6%
50-59	18%	16%	16%
60-69	35%	36%	36%
70-79	11%	29%	27%
80 or older	0.2%	12%	11%
Sex			
Female	47%	55%	54%
Male	53%	45%	46%

as well as the occurrence of hypoglycemic episodes. All the questionnaires and the CATI data were coded with unique, anonymous identifiers, which allowed the data from different sources to be linked.

The characteristics of the random sample of patients with DM in the study

A random-quota sampling was used to obtain at least 60 patients with type-1 DM described by the GPs for each of the three types of locations and at least 120 patients with type-1 DM described by the Ds for each of the two types of locations. In total, a sample of 2032 patients with DM was obtained, 96.8% of the planned sample size. A complete set of data (the medical questionnaire completed by the physician and the costs questionnaire completed by the patient) was obtained for 1938 DM patients and it was put into the survey datafile for further analysis. The age and gender structure of survey participants is shown in Table 1: females accounted for 52% and males for 48% of the sam-

ple. 1532 patients with DM agreed to take part in the CATI. Projected data is shown in Table 1a.

Data analysis

In order to estimate the prevalence of selected conditions in the general DM patient population, multi-dimensional

data weighting was carried out on the sample. The following factors were taken into account in designing the weights:

- data on the number of physicians – weights were used to adjust the proportion between the number of GPs and Ds, on the basis of HDM data on the general population of physicians in Poland [6];
- data on the average number of all patients seen daily, weekly and monthly by the GP or D based on a two-week detailed register compiled by the physicians during the study;
- data on the physicians' average number of working days in a month, adjusted for days taken off from work and holidays (based on two-weeks of detailed registers) [7-8];
- data on the frequency of patients' visits to the GPs or Ds based on average intervals between visits.

Weights used in the *Economedica* survey were obtained using:

1. Data from NFZ for the randomly selected sample of DM patients in Poland within each of the 16 provinces. 384000 patients, who were > 18 years of age in 2017, were randomly selected from a database of 37.97 million PESEL numbers (Polish national ID numbers – Powszechny Elektroniczny System Ewidencji Ludności). For the 384,000 PESEL numbers, the NFZ obtained information about all the medical procedures and products (e.g. drugs, medical devices, nutritional products) financed by the NFZ. The anonymised data was forwarded to the National Institute of Public Health (NIZP – Narodowy Instytut Zdrowia Publicznego), which conducted the analysis.
2. NFZ datafile on the volume of drugs reimbursed in pharmacies on the basis of prescriptions [9].
3. NFZ data on the structure of the DM patient population according to age, gender and type of DM [4].
4. Data on the number of packs of drugs used in the treatment of DM, prescribed by GPs and Ds in Poland in 2016, which was obtained from PEX PharmaSequence's RECEPTOmetr service. RECEPTOmetr is a commercially available, on-going database service for monitoring medical prescriptions, which used a monthly random survey among approximately 10 million medical prescriptions. In 2016 the data was collected from a sample of 4300 pharmacies, out of an estimated total of 14500 pharmacies

in Poland, proportionally distributed across 16 provinces and types of locations. The patient age structure in the RECEPTOmetr database is in line [+/- 0.8%] with the data reported by the NFZ. The age structure of patients with type-2 DM was adjusted based on the PEX PharmaSequence's RECEPTOmetr prescription data.

Results

The weighted structure of the population of DM patients' according to the place of residence is shown in Table 2. While every 3rd patient lived in a location where the population was < 20000, every 5th was a resident of the largest cities in Poland. Regardless of their place of residence, we observed that the patients included in the study more often visited physicians who work in large cities.

The distribution of the pharmacotherapy patterns of DM in the examined random sample of adults is presented in the Table 3, while weighted results are shown in the Table 3a. The projected data indicates that DM patients are predominantly treated only with oral drugs (67% of females and 60% of males). 19% of the female patients in the study and the same proportion of males were treated with a combination of oral drugs and insulin, while the remaining patients (14% of females and 21% of males) were treated only with insulin.

Weighted results show that the group of patients with type-1 DM is dominated by those whose disease started before the age of 40 (70%). In contrast, the group of patients with type-2 DM, the majority (76%) are patients whose DM was diagnosed after the age of 50 (Table 4).

On the basis of the physicians' registers of patient visit over a period of two weeks, we estimated that patients with

Table 2. DM patients' population by the patient's place of residence

Size of the place of residence	Number of patients	% of the sample	% of population (weighted data)
Patient's place of residence			
village	447	23.1%	20.9%
town < 20,000	200	10.3%	11.1%
city from 20,000 to 100,000	332	17.1%	17.2%
city from 100,001 to 200,000	99	5.1%	4.6%
city from 200,001 to 500,000	280	14.4%	15.2%
city 500,001 or more	362	18.7%	19.8%
NA	218	11.2%	11.2%

N – number of individual patients

Table 3. DM patients' sample structure according to the way of DM treatment (raw data)

Patient population	Only insulin		Only oral drugs		Insulin + oral drugs		Total	
	N	% of the sample	N	% of the sample	N	% of the sample	N	% of the sample
Sex								
female	251	25%	596	59%	159	16%	1006	52%
male	271	29%	514	55%	147	16%	932	48%

N – number of individual patients

Table 3a. DM patients sample structure according to the way of DM treatment (weighted data)

Patient population	Only insulin	Only oral drugs	Insulin + oral drugs	Total
	% of the sample	% of the sample	% of the sample	% of the sample
Sex				
female	14%	67%	19%	54%
male	21%	60%	19%	46%

DM accounted for 16% of all the patients seen by GPs and 88% of all the patients seen by Ds (Table 5). In the whole examined sample, 87.5% were patients with type-2 DM. The average (per patient) monthly medical costs directly incurred by patients related to DM and/or DM-related complications reached 292.5 PLN (66.2 EUR) (Table 6, weighted data). The costs of the medications prescribed by doctors reached 42.8% of the patients' total medical expenses. The average (per patient) monthly non-medical costs directly incurred by patients and that were related to DM and/or DM related complications reached 162.7 PLN (36.8 EUR) (Table 7, weighted data). These costs were dominated by additional food expenses due to illness-related diet, which amounted to 60% of the patients' non-medical costs.

Table 4. Distribution of DM patients according to the type of DM and patient's age at the time of DM diagnosis

Patient's age at the time of DM diagnosis	Type 1 diabetes	Type 2 diabetes
0-9	4%	0%
10-19	17%	0%
20-29	27%	2%
30-39	22%	5%
40-49	11%	16%
50-59	13%	31%
60-69	5%	28%
70 +	0%	15%
NA	1%	3%

NA – data non available

Discussion

The *Economedica* study was primarily designed to broaden the scope of current data related to the diagnosis and treatment of individuals suffering from DM in Poland. In many countries around the world, epidemiological data on

morbidity and prevalence is neither complete nor systematically collected. This is the case also in Poland. Although several important studies have been carried out and many articles on the epidemiology of DM patients have been published in recent years, the epidemiological situation is still not well described. It is important to note the value of studies such as NATPOL and WOBASZ, which estimate the

Table 5. Average number of patients admitted by the physician per week, including the percentage of patients diagnosed with DM (raw data)

	General Practitioners		Diabetologists	
	Average number of patients seen per week	% of the sample	Average number of patients seen per week	% of the sample
Patients, total	170	100%	100	100%
Patients with DM	26	16%	89	88%
Type-1 DM	3	2%	12	12%
Type-2 DM	23	14%	77	77%

Table 6. Average monthly medical costs per patient – costs directly incurred by patients related to DM and/or DM related complications (weighted data) as declared by the patients

Cost category	Monthly average expense (n = 1724)	
	PLN	EUR
Medications prescribed by a doctor	125.2	28.3
In-patient treatment (hospital, sanatorium)	46.0	10.4
Medical materials and equipment	34.9	7.9
Over-the-counter medications not recommended by a doctor	31.8	7.2
Paid medical visits	25.3	5.7
Rehabilitation, physiotherapy	12.3	2.8
In-patient treatment (private healthcare)	7.0	1.6
Diagnostic tests, check-ups	6.9	1.6
Consultation with a psychologist, psychotherapist	1.6	0.4
Paid nursing care	1.5	0.3
Other medical expenses	0.0	0.0
Sum of average medical expenses	292.5	66.2

N – number of patients

Adopted euro exchange rate – 4.42 PLN (2016.12.30)

current incidence of DM in Poland at 6.7%-8.4% [5, 10-12]. Thanks to the fact that these studies were repeated regularly over 10 years, they provide a meaningful picture of the changes that have taken place over this time. Nonetheless, these epidemiological studies only create the foundations for knowledge about DM by describing the prevalence of the disease. There is still a lack of up-to-date data that reflects a broad range of epidemiological factors on DM complications, which can account for a significant cost both for public finances and for patients [13-14].

The study presented in this article aimed to provide the most detailed description of the selected sample of DM patients with attention to the differences between cases of type-1 and type-2 DM. Since DM-related complications occur at a different frequency in each of the two types of DM and the disease-related costs can also differ, we planned to test

a sufficiently high number of patients with type-1 DM – 500 individuals, which constituted 24% of all the respondents in the study. It should be noted that this percentage does not correspond to the prevalence of type-1 DM among all DM patients. Nonetheless, it was already assumed at the planning stage of the study that we would adjust for the frequency of estimated complications in the two patient groups by referencing the most recent published epidemiological data.

Although the main goal of our study was to evaluate the prevalence of DM-related complications and to obtain data on the costs related to treatment of DM, the data collected in the study show that having applied the weights and adjustments described in the methodology, the age and gender structure of the patients participating in our survey does not significantly differ from the age and gender structure of the general DM patient population in Poland in 2013,

Table 7. Average monthly non-medical costs per patient (costs directly incurred by patients related to DM and/or DM related complications), as declared by the patients

Cost category	Monthly average expense (n = 1724)	
	PLN	EUR
Additional food expenses due to illness-related diet	97.6	22.1
Transportation expenses incurred by the patient and accompanying persons in connection with treatment	29.9	6.8
Additional expenses for cosmetics and clothing due to illness	23.2	5.2
Additional home help due to the inability of the patient or their caregivers	8.4	1.9
Providing care for children/other people under the care of the patient due to the absence of the patient or his/her caregivers	1.8	0.4
Accommodation expenses incurred by the patient and accompanying persons in connection with treatment	1.2	0.3
Other non-medical expenses	0.5	0.1
Sum of average non-medical expenses	162.7	36.8

N – number of patients

Adopted euro exchange rate – 4.42 PLN (2016.12.30)

as described by Topór-Mądry et al. [4]. The prevalence of DM increases with age and DM dominates older patient groups. This distribution coincides with observations from other European studies [15-16]. On the other hand, there are more females in the population of the study in Poland, which differs from the data presented by the International Diabetes Federation (IDF) where the prevalence of DM is higher among males [16]. This may result from the fact that only those who are aware of their disease were included in the study. Data in the IDF's Diabetes Atlas presents estimates of DM prevalence. This data also takes into account the general lack of awareness about DM, which in Poland is greater among men [10-11].

It is important to emphasize that this study is not based on a random, representative sample of all patients suffering from DM in Poland. Instead, it should be treated as a description of the population of patients seeking medical advice concerning DM from GPs and Ds. In Poland diabetological care is predominantly the domain of these two medical specialists and the RECEPTometr data for 2017 shows that 84.1% of drugs packs from the A10 group were prescribed by GPs and Ds.

Additionally, observations from this study show that the percentage of DM patients' visits to GPs is high in relation to morbidity. DM patients' visits accounted for 16% of all visits to GPs. This clearly indicates a high workload for this group of physicians connected to managing DM patients. Determining what percentage of these patients have uncontrolled DM is an important task in terms of healthcare system management in Poland. Potential measures aimed at improving

the control of DM may involve reducing the workload of GPs, especially that a general shortage of GPs is consistently observed throughout the country.

It is worth noting that DM creates a heavy financial burden on patients. The results of this study indicate that the average monthly out-of-pocket medical expenditures made by the patient reached almost 300 PLN (mainly for prescription drugs, which amount to 60% of the total medical costs). After adding the non-medical costs, the total amount was to close to 450 PLN monthly. The majority of the surveyed patients were over the age of 60, thus such costs can easily reduce their patients' adherence to the treatment.

Conclusions

The diagnosis and treatment of DM causes a heavy burden on public finances, also in terms of social insurance [17-21]. These costs significantly increase when there is a dynamic increase in the number of newly diagnosed DM patients. Although financing the DM diagnostic process and treatment is an increasing challenge, particularly due to the limited public funds earmarked for public health in Poland, a detailed cost structure (including the costs associated with DM-related complications and the different courses of concomitant diseases) is yet to be thoroughly explored [22-23].

As in the case of many other countries, there is no register of patients with DM in Poland, therefore the *Economedia* study can be used as a model to monitor the most important epidemiological, therapeutic, and cost trends among

patients affected by DM. We agree that further detailed analysis of the data from this survey, coupled with the data obtained from the NFZ by the NIZP, will enable an extended assessment of the prevalence of DM and its complications. It will also allow for the calculation of the additional burden on NFZ budget, which results from different courses of concomitant diseases in patients with DM as compared to patients not diagnosed with DM.

Hygiene and the DM Epidemiology Group from the Public Health Committee of the Polish Academy of Sciences. The study was financed by Sequence (now PEX PharmaSequence Sp. z o.o.)

Acknowledgements

The study was carried out by Sequence Sp. z o.o (now PEX PharmaSequence Sp. z o.o.) in co-operation with the National Institute of Public Health – National Institute of

Funding

None.

Conflicts of interest

There is no conflict of interest in this project.

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Del Nido cardioplegia versus other contemporary solutions for myocardial protection – a literature review

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Abstract

Myocardial protection is one of the most important factors ensuring patient safety during cardiac surgery with the application of cardiopulmonary bypass. Infusion of cardioplegic solution into the coronary circulation protects the heart and provides a standstill operating field for the surgeon. Cold blood cardioplegia and crystalloid cardioplegia are the two main types of solutions with a long history of use and a large amount of research proving their efficacy and safety. Relatively new del Nido cardioplegia seems to be an interesting alternative. We reviewed the literature comparing del Nido cardioplegia with two other types of cardioplegic solutions. We took into consideration many different clinical and biochemical aspects may indicate the quality of cardioprotection.

Keywords: cardiac surgery · cardioprotection · del Nido cardioplegia · cold blood cardioplegia · Bretschneider HTK cardioplegia

Citation

Brzeska B, Karolak W, Żelechowski P, Łoś A, Ulatowski N, Pawlaczyk R. Del Nido cardioplegia versus other contemporary solutions for myocardial protection – a literature review. Eur J Transl Clin Med. 2023;6(1):41-57.

DOI: [10.31373/ejtcmm/163317](https://doi.org/10.31373/ejtcmm/163317)

Introduction

Effective myocardial protection is crucial for a successful outcome of cardiac surgery with the application of cardiopulmonary bypass (CPB). For nearly 50 years the most common method of cardioprotection has been the infusion

of cardioplegic solution into the coronary vessels. This provides a bloodless and motionless operating field which is perfect for precise surgery, however with a time limit resulting from the principles of physiology and biochemistry. The safe ischemic time varies depending on the composition and temperature of the cardioplegic solution. Some of the warm

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Available online: www.ejtcmm.gumed.edu.pl

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blood infusions must be delivered continuously, whereas a single infusion of some cold crystalloid solutions provide over two hours of secure myocardial protection during cross-clamping (XC) of the aorta. Thanks to research we have a much better understanding of the mechanism of ischemia-reperfusion injury (IRI) and better ways to prevent it.

Cardioplegia is a name given to a group of solutions using different mechanisms to arrest the heart in diastole with additives for cell membrane stabilization or oedema prevention. The mechanism of each is the result of a characteristic composition (Table 1). The development of high-potassium blood cardioplegia was a milestone in cardiac surgery. However, short intervals between doses are its weak point, particularly in the current era of minimally invasive cardiac procedures. Whereas the low-sodium crystalloid solution provides a very long working interval and rarely has to be re-dosed, however the long reperfusion time is a disadvantage. Del Nido cardioplegia (DNC) solution derived from pediatric cardiac surgery combines the most important aspects of the high-potassium and low-sodium cardioplegia solutions

Table 1. Cardioplegia solutions composition

Component	DNC	HTK	CBC
Na ⁺ (mmol/l)	150	15	136-152
Cl ⁻ (mmol/l)	132	50	126-132
K ⁺ (mmol/l)	24	9	13-24
Mg ²⁺ (mmol/l)	6	4	2-13
Ca ²⁺ (mmol/l)	0.4	0.02	0-1
Lidocaine (mg/l)	140	-	27
Mannitol (mmol/l)	14.5	30	0-12
Osmolality (mOsmol/kg)	294	300	304-320
Ketoglutarate (mmol/l)	-	1	-
Tryptophan (mmol/l)	-	2	-
Histidine (mmol/l)	-	198	-

CBC – cold blood cardioplegia, DNC – Del Nido cardioplegia, HTK – Bretschneider histidine-tryptophan-ketoglutarate solution

making it an interesting alternative for adult patients. Despite the growing number of studies and meta-analysis, there are no guidelines or consensus regarding which cardioplegic solution is the most beneficial.

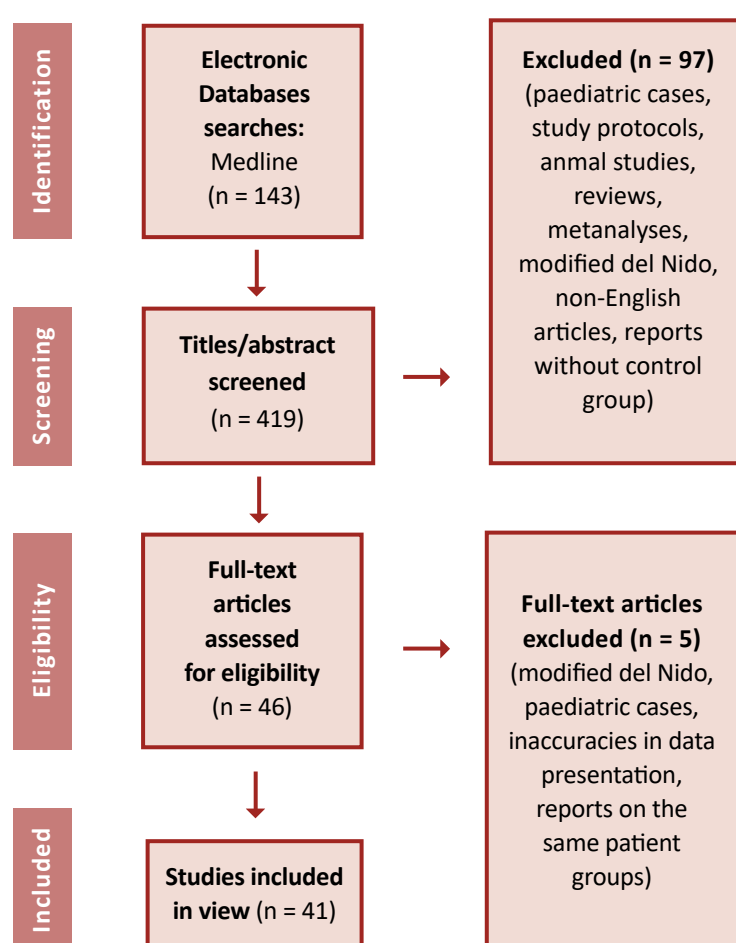


Figure 1. Literature search flow chart

Material and methods

For this review, we included retrospective and prospective studies comparing DNC with cold blood cardioplegia (CBC) of any kind or crystalloid cardioplegia (Bretschneider histidine-tryptophan-ketoglutarate solution (HTK)). We searched the MEDLINE database for English-language articles using the following keywords: “del Nido cardioplegia,” “del Nido vs cardioplegia” and “cardioplegia AND del Nido”. We looked for articles on standard adult cardiac procedures (single valve or coronary artery bypass grafting (CABG)) and combined procedures (multivalve, reoperations, aortic root). After evaluation of the substantive value of abstracts, full-text articles were analyzed. The analyzed studies concerned a total of 9839 cases, including 5073 with del Nido cardioplegia. Experimental, animal-model, pediatric and modified del Nido studies were excluded (Figure 1). Results from selected studies are collected and presented in the form of tables with an indication of the number of included patients, the type of surgery and study design (prospective, retrospective and retrospective with propensity-score matching (PSM)).

Results

Cardiopulmonary bypass and cross-clamp times

Significant shortening of the CPB and XC times in the DNC groups compared with CBC was described in most of the analyzed studies [1-17] (Table 2). Four studies [18-21]

reported shorter XC times without a change in the CPB time. This may confirm that surgery is performed smoothly when DNC is used but may suggest the necessity of a longer reperfusion or lidocaine residual effect with delayed activation of the conductive system. Compared with HTK, there were no reported differences in CPB or XC times. This might be due to the fact that only one dose of cardioplegia was usually needed in both groups.

Table 2. CPB and XC times

Article	Cardio- plegia	Study design	Cardiac procedures	No. of pa- tients	CPB times [min]			p value	Cross clamp times			p value
					DN	BC	HTK		DN	BC	HTK	
Eris (2021)	DN vs BC	retro- specti- ve	aortic root proce- dures	118	114.4 ±27.1	123.9 ±28.8	-	0.071	73.3 ±20.7	87.5 ±23.2	-	0.001
Kagan (2021)	DN vs BC	retro- spective	CABG	350	90.28 ±25.56	93.56 ±24.78	-	0.175	57.75 ±16.96	63.56 ±18.45	-	0.014
Lama (2021)	DN vs BC	RCT	CABG	90	106.13 ±24.65	107.62 ±18.69	-	0.02	66.22 ±15.39	72.07 ±12.23	-	0.04
Lenoir (2021)	DN vs BC	PM	aortic root procedures	110	163±5	181±5	-	0.01	145±4	161±4	-	0.006
Reidy (2021)	DN vs BC	retro- spective	CABG in veterans	155	96.8 ±32.1	117.0 ±27.3	-	< 0.01	63.9 ±21.8	71.7 ±18.6	-	0.02
Ross (2021)	DN vs BC	retro- spective	all cardiac procedures	497	96 (73-121)	96 (73-127)	-	0.82	67 (48-92)	63 (48-90)	-	0.63
Sanri (2021)	DN vs BC	retro- spective	CABG	255	95.16 ±23.48	90.01 ±24.25	-	0.086	59.30 ±17.36	62.80 ±18.66	-	0.042
Sevuk (2021)	DN vs BC	PM	all cardiac procedures and XC time > 90 min	176	161.0 (153.2- 170.7)	163.5 (156.0- 175.5)	-	0.3	99.0 (94.2- 106.0)	124.0 (117.0- 131.0)	-	< 0.001
Shu (2021)	DN vs BC	PM	all cardiac procedures	326	111.0 (90.5- 130.0)	106.0 (85.0- 134.0)	-	0.422	75.0 (58.5- 90.5)	73.0 (57.5- 91.5)	-	0.564
Algarni (2020)	DN vs BC	retro- spective	all cardiac procedures	305	150±51	178±60	-	< 0.001	118±42	143 ±60	-	< 0.001
Chen (2020)	DN vs BC	PM	acute aortic dissection	122	165.0 ±12.5	168.0 ±10.5	-	0.154	93.2 ±9.5	91.8 ±9.0	-	0.405
George (2020)	DN vs BC	PM	CABG or valve	286	90 (74-116)	87 (76-108)	-	0.516	59 (48-75)	57 (47-70)	-	0.65
Kirisci (2020)	DN vs BC	RCT	CABG	60	95.07 ±23.06	114.13 ±33.93	-	0.01	57.30 ±23.57	76.07 ±27.18	-	0.01
Kuserli (2020)	DN vs BC	PM	aortic root procedures	72	129.39 ±5.72	151.94 ±10.12	-	0.001	96.75 ±5.24	116.42 ±9.27	-	0.001
Orak (2020)	DN vs BC	retro- spective	elective all cardiac pro- cedures	83w	114.58 ±6.30	112.43 ±6.40	-	0.811	77.93 ±6.12	70.73 ±6.17	-	0.41
Schutz (2020)	DN vs BC	PM	CABG or CABG and valve	670	51.600 ±20.625	49.233 ±22.230	-	0.109	32.063 ±15.795	31.313 ±16.384	-	0.513
Sharma (2020)	DN vs BC	retro- spective	AVR + MVR	209	92.1 ±12.3	129.5 ±11.0	-	0.0001	72.6 ±10.2	98.2 ±9.2	-	0.0001

Table 2. continuation

Article	Cardio-plegia	Study design	Cardiac procedures	No. of patients	CPB times [min]			p value	Cross clamp times			p value
					DN	BC	HTK		DN	BC	HTK	
Ucak (2020)	DN vs BC	retro-spective	AVR	137	53.7 ±15.2	60.8 ±18.5	-	0.046	41.5 ±11.8	48.7 ±12.3	-	0.041
Willekes (2020)	DN vs BC	PM	aortic root procedures	344	189±68	245±92	-	<0.0001	132±53	186 ±74	-	<0.0001
Kuciński (2019)	DN vs BC	retro-spective	all cardiac procedures	2108	65 (50-85)	55 (42-72)	-	<0.001	40 (29-59)	33 (24-47)	-	<0.001
Luo (2019)	DN vs BC	PM	MI AVR/ MVR/DVR	132	98.00 (77.50-129.75)	105.50 (86.00-128.00)	-	0.428	56.50 (45.25-80.00)	61.50 (46.00-77.00)	-	0.368
Pragliola (2019)	DN vs BC	PM	all cardiac procedures	204	-	-	-	-	108±52	106±44	-	0.3
Sanetra (2019)	DN vs BC	RCT	AVR	150	67.96 ±20.46	69.25 ±19.23	-	0.564	55.18 ±15.93	55.52 ±14.03	-	0.419
Timek (2019)	DN vs BC	PM	CABG	650	85±26	99±28	-	<0.01	68±20	82±24	-	<0.01
Kim (2018)	DN vs BC	PM	valve procedures	208	99.1 ±39.4	155.2 ±62.4	-	<0.001	75.3 ±31.7	102.1 ±47.3	-	<0.001
Koeckert (2018)	DN vs BC	PM	MI AVR	118	77.6 ±27.3	74.8 ±16.5	-	0.492	58.1 ±17.7	56.6 ±11.8	-	0.591
Sabik (2018)	DN vs BC	PM	MI MVP	124	72.3 ±19.0	94.2 ±20.7	-	<0.0001	51.3 ±15.7	66.4 ±16.8	-	<0.0001
Ad (2017)	DN vs BC	RCT	CABG and/or single valve	89	97	103	-	0.288	70	83	-	0.018*
Hamad (2017)	DN vs BC	retro-spective	AVR + CABG	50	65.5 ±12.5	76.6 ±19.1	-	0.019	55.6 ±11.2	64.3 ±18.9	-	0.05
Ziazadeh (2017)	DN vs BC	PM	MI AVR	126	108±24	135±43	-	Sign	80±16		-	Signifi- cant
Kim (2016)	DN vs BC	PM	all cardiac procedures	78	158±68	162±58	-	0.786	97±42	101±41	-	0.662
Mischra (2016)	DN vs BC	retro-spective	CABG and DVR	100	158.60 ±39.92	179.81 ±42.36	-	0.041	110.15 ±36.84	133.56 ±35.66	-	0.012
Vistarini (2016)	DN vs BC	retro-spective	MI AVR	46	67±27	59±24	-	0.19	56±13	48±17	-	0.07
Ota (2015)	DN vs BC	PM	AVR	108	71±16	84±28	-	0.003	52±14	60±16	-	0.004
Ramanathan (2015)	DN vs BC	retro-spective	all cardiac procedures	142	105.0	109.5	-	0.5862	71.3	72.0	-	0.9038
Sora-bella (2014)	DN vs BC	retro-spective	reoperative AVR	113	61.3 ±18.9	67.2 ±20.1	-	0.111	93.1 ±25.7	100.5 ±34.3	-	0.204
Duan (2021)	DN vs HTK	PM	complex valve surgery	146	149 (116-181)	-	158 (126-187)	0.232	107 (74-134)	-	100 (83-133)	0.795
Gunaydin (2020)	DN vs HTK	RCT	MI AVR	200	-	-	-	-	99±27	-	102 ±33	0.7
Lee (2020)	DN vs HTK	PM	MICS	456	112.5 ±44.8	-	115.8 ±36.0	0.386	74.8 ±28.6	-	77.1	0.081
Mehra-banian (2018)	DN vs HTK	RCT	complex cardiac procedures	40	103.19 ±23.42	-	97.36 ±16.70	0.376	73.76 ±19.66	-		0.083

* Not significant due to alpha < 0.001

Table 4. Rate of electric defibrillation

Article	Year	Cardioplegia	Study design	Cardiac procedures	No. of patients	Defibrillation %			p value
						DN	BC	HTK	
Eris (2021)	2021	DN vs BC	retrospective	aortic root procedures	118	44.4	69.2	-	0.006
Lama (2021)	2021	DN vs BC	RCT	CABG	90	15.55	24.44	-	0.42
Ross (2021)	2021	DN vs BC	retrospective	all cardiac procedures	497	8	23	-	< 0.001
Sevuk (2021)	2021	DN vs BC	PM	all cardiac procedures and XC time > 90 min	176	10.2	15.9	-	0.26
Chen (2020)	2020	DN vs BC	PM	acute aortic dissection	122	11.3	46.7	-	< 0.001
Kirisci (2020)	2020	DN vs BC	RCT	CABG	60	10.00	6.67	-	0.64
Kuserli (2020)	2020	DN vs BC	PM	aortic root procedures	72	22.2	52.8	-	0.007
Sharma (2020)	2020	DN vs BC	retrospective	AVR + MVR	209	21.2	65.9	-	0.00001
Ad (2017)	2017	DN vs BC	RCT	CABG and/or single valve	89	4.7	13.2	-	0.244
Hamad (2017)	2017	DN vs BC	retrospective	AVR + CABG	50	20	60	-	0.009
Mischra (2016)	2016	DN vs BC	retrospective	CABG and DVR	100	4	4	-	1.000

Cardiac enzymes

In the majority of analyzed articles, the postoperative levels of cardiac enzymes (creatine phosphokinase myocardial band (CK-MB), troponin T or I (TnT and TnI), depending on study protocol) were similar or lower when DNC was compared with CBC cases (Table 3; [download here](#)). Different results were shown in the subgroup analysis of aortic root cases [2] with XC time > 180 min where CK-MB levels were significantly higher in the DNC group. Significantly higher postoperative TnT levels in the DNC group were reported also in a retrospective study of 2108 cardiac cases of all types [10]. However, a more thorough analysis revealed that uneven distribution of surgery types (resulting in longer CPB and XC times in DNC) and preoperative TnT level was responsible for that result. In two studies comparing DNC with HTK cardiac enzymes were comparable [22-23] between the groups.

Two other studies on DNC vs. HTK suggested a higher level of cardiac enzymes in cases when DNC was used with longer XC. Lee et al. [24] showed lower peak TnI and CK-MB in DNC. A peak level of cardiac enzymes according to the XC time was also lower in DNC, but the differences between groups tended to decrease with a crossover point of ~100 minutes of XC. In a sub-analysis of cases with a second cardioplegia dose, Gunaydin et al. showed significant differences in TnT levels measured during weaning from CPB to the disadvantage of DNC [25].

Cardiac activity and return to sinus rhythm

The quality of myocardial protection may be also estimated by observing the electrical and mechanical activity of the arrested heart: satisfactory protection is shown by no such activity before the clamp removal and reperfusion. Ucak et al. [8] reported ventricular fibrillation (VF) during XC in 5.7% of cases in DNC and 9.4% in the CBC group ($p = 0.016$). In the RCT by Sanetra et al. [26] no differences between groups in terms of electrical activity during XC were observed. In majority of the studies, patients with DNC more often had a spontaneous return of sinus rhythm (SR) return and less often needed electric defibrillation, compared to those with CBC (Table 4). When compared with HTK, only one study [23] reported a rate of spontaneous SR return. It was significantly higher in the DNC group and even more pronounced in a subgroup of cases with XC > 120 min (93.1% vs 42.3%). A very high number of VF in the HTK groups raises some questions.

Cardioplegia volume and blood product transfusion rates

In the majority of reviewed studies, total cardioplegia volume was significantly higher in the CBC than in the DNC cases [1, 4-6, 8-9, 11, 15, 17-18, 20, 26-33]. In only one study this volume was lower in CBC patients [21]. When only crystalloid volume is taken into consideration, higher volume was administered to patients who received DNC [1, 14, 31,

34-35]. The differences in administered volume did not seem to affect the postoperative hematocrit (HCT) levels. This parameter was analyzed in 10 articles and 6 of them reported comparable HCT levels between DNC and CBC [9, 11, 27-28, 31, 34]. In the other four studies, HCT was higher in the DNC group [6, 14, 18, 36].

The amount of transfused blood and blood products was similar in the majority of studies comparing DNC and CBC. Due to the lack of homogeneity in reporting between articles in Table 5, we present only data about red blood cell (RBC)

transfusions. Three studies [6, 18, 34] showed a higher need for different kinds of blood products in the CBC group. When DNC was compared with HTK, haemoglobin levels as well as the rate of transfusions during CPB and in the postoperative period were comparable between the groups [22, 24-25]. Only Duan et al. [23] reported a higher need for platelet concentrates (PC), fresh frozen plasma and cryoprecipitate in the HTK group. In the subgroup analysis of cases with XC > 120 minutes, only differences in PC remain significant.

Table 5. RBC transfusions

Article	Cardio-plegia	Study design	Cardiac procedures	No. of patients	RBC transfusions			p value
					DN	BC	HTK	
Lenoir (2021)	DN vs BC	PM	aortic root procedures	110	22%	26%	-	0.65
Reidy (2021)	DN vs BC	retro-spective	CABG in veterans	155	25.7%	17.6%	-	0.41
Ross (2021)	DN vs BC	retro-spective	all cardiac procedures	497	37%	35%	-	0.61
Sevuk (2021)	DN vs BC	PM	all cardiac procedures and XC time > 90 min	176	0.5 (0.0-1.0) U	1.0 (0.0-1.0) U	-	0.72
Kuserli (2020)	DN vs BC	PM	aortic root procedures	72	0.47±0.74 U	1.75±0.97 U	-	0.001
Orak (2020)	DN vs BC	retro-spective	elective all cardiac procedures	83	76.6%	75.7%	-	0.87
Schutz (2020)	DN vs BC	PM	CABG or CABG and valve	670	1.97 U	1.67 U	-	0.45
Sanetra (2019)	DN vs BC	RCT	AVR	150	24.0%	29.3%	-	0.56
Koeckert (2018)	DN vs BC	PM	MI AVR	118	0.81±2.41 U	0.46±1.24 U	-	ns
Ad (2017)	DN vs BC	RCT	CABG and/or single valve	89	4%	7%	-	0.658
Hamad (2017)	DN vs BC	retro-spective	AVR + CABG	50	0.68±0.95 U	1.32±1.89 U	-	0.136
Ziazadeh (2017)	DN vs BC	PM	MI AVR	126	0.24±0.80 U	0.24±0.56 U	-	ns
Vistarini (2016)	DN vs BC	retro-spective	MI AVR	46	4%	48%	-	0.006
Sorabella (2014)	DN vs BC	retro-spective	reoperative AVR	113	1.4±1.7 U	2.1±2.6 U	-	0.07
Duan (2021)	DN vs HTK	PM	complex valve surgery	146	0.75 (0.00-4.00) U	-	0.00 (0.00-4.00) U	0.682
Gunaydin (2020)	DN vs HTK	RCT	MI AVR	200	1.0±0.5 U	1.0±0.5 U	-	0.8
Lee (2020)	DN vs HTK	PM	MICS	456	893±1290 mL	-	907±1228 mL	0.905
Mehrabanian (2018)	DN vs HTK	RCT	complex cardiac procedures	40	38.1%	-	42.1%	0.525

Table 6. Postoperative AF occurrence

Article	Cardio- plegia	Study design	Cardiac procedures	No. of pa- tients	Atrial fibrillation [%]			p value
					DN	BC	HTK	
Eris (2021)	DN vs BC	retrospective	aortic root procedures	118	11.1	12.3	-	0.84
Lenoir (2021)	DN vs BC	PM	aortic root procedures	110	27	18	-	0.27
Reidy (2021)	DN vs BC	retrospective	CABG in veterans	155	30.0	24.7	-	0.46
Sanri (2021)	DN vs BC	retrospective	CABG	255	24.2	35.8	-	0.044
Sevuk (2021)	DN vs BC	PM	all cardiac procedures and XC time >90 min	176	21.6	29.5	-	0.22
Shu (2021)	DN vs BC	PM	all cardiac procedures	326	20.86	22.70	-	0.788
Algarni (2020)	DN vs BC	retrospective	all cardiac procedures	305	14.7	17.6	-	0.56
George (2020)	DN vs BC	PM	CABG or valve	286	7.7	6.3	-	0.643
Kuserli (2020)	DN vs BC	PM	aortic root procedures	72	22.2	25.0	-	0.781
Orak (2020)	DN vs BC	retrospective	elective all cardiac procedures	83	20.9	17.5	-	0.692
Schutz (2020)	DN vs BC	PM	CABG or CABG and valve	670	22.7	31.0	-	0.018
Ucak (2020)	DN vs BC	retrospective	AVR	137	28	24	-	0.71
Willekes (2020)	DN vs BC	PM	aortic root procedures	344	31.4	36.0	-	
Sanetra (2019)	DN vs BC	RCT	AVR	150	24	21	-	0.71
Timek (2019)	DN vs BC	PM	CABG	650	33	23	-	< 0.01
Koeckert (2018)	DN vs BC	PM	MI AVR	118	37.3	27.1	-	0.162
Sabik (2018)	DN vs BC	PM	MI MVP	124	18	28	-	0.18
Hamad (2017)	DN vs BC	retrospective	AVR + CABG	50	44	36	-	0.773
Ziazadeh (2017)	DN vs BC	PM	MI AVR	126	33	22	-	ns
Kim (2016)	DN vs BC	PM	all cardiac procedures	78	35.9	30.8	-	0.804
Mischra (2016)	DN vs BC	retrospective	CABG and DVR	100	30.00	16.67	-	0.22
Ota (2015)	DN vs BC	PM	AVR	108	37.0	33.3	-	0.84
Ramanathan (2015)	DN vs BC	retrospective	all cardiac procedures	142	31	31	-	ns
Gunaydin (2020)	DN vs HTK	RCT	MI AVR	200	18.9	-	11.5	0.035

Heart rhythm disturbances in the postoperative period

The majority of reviewed articles showed comparable results between DNC and CBC in terms of postoperative atrial fibrillation (AF) occurrence (Table 6). Only Timek et al. [11] reported a higher rate of AF after isolated CABG with DNC. Opposite results with a higher rate of AF in CBC cases were shown in the two studies. The first of which [20] showed a correlation between the occurrence of AF and cardioplegia volume in patients who underwent isolated CABG. In the second study, [37] patients who underwent CABG with or without valve intervention had a higher rate of AF, atrial flutter and VT in cases with CBC in all analyses excluding subgroups with left ventricular ejection fraction (LVEF) < 35%.

Shu et al demonstrated a comparable rate of AF but higher risk of ventricular rhythm disturbances was shown in

PSM analysis as well as in sub-analysis of cases with XC > 69 minutes [38]. In log regression analysis DNC had a significant effect on reduction in the rate of ventricular arrhythmias.

The need for a temporary pacemaker was equal [39] or higher in DNC groups [40] when compared with CBC.

Two studies comparing DNC with HTK presented results on postoperative arrhythmia. Gunaydin et al. [25] reported a higher rate of AF and atrioventricular (AV) block in the general cohort and subgroup with a second cardioplegia dose when DNC was used. When only one dose was administered, the results were comparable. Different results were reported by Duan et al. [23]. 3rd degree AV block, VF and sudden cardiac arrest were more frequent in the HTK group. However, in the logistic regression model, the type of cardioplegia did not have a statistically significant influence on the occurrence of severe post-operative arrhythmia. No differences were found in a sub-analysis of cases with XC > 120 minutes.

Inotropic/vasoactive support and low cardiac output syndrome

Due to heterogeneity in reporting it is difficult to show and analyze differences in the need for inotropic/vasopressor support. Only Orak et al. [27] report lower noradrenaline (NA) requirements during surgery when CBC was used. Seven of reviewed articles showed a lower need for inotropic/vasopressor support in the DNC group [2, 4, 7-8, 11, 15, 18]. In Ad et al. [31] the need for inotropic support was also lower in the DNC group, but significance was not reached due to an alpha level of $p < 0.001$. A total of 19 other articles

reported no differences in terms of the need for inotropic/vasoactive and rate of low cardiac output syndrome (LCOS) between DNC and CBC. Three [22, 24-25] of a total of four studies comparing DNC and HTK showed no differences in the need for inotropic support. Only in one sub-analysis of cases with XC < 120 minutes from the study by Duan et al. [23] the doses of NA were lower in the DNC group.

Non-cardiac or systemic complications

Ross et al. [30] showed no differences in the rate of acute kidney injury (AKI), stroke or composite death. However, the

Table 7. Early mortality

Article	Cardio-plegia	Study design	Cardiac procedures	No. of patients	RBC transfusions			p value	info
					DN	BC	HTK		
Eris (2021)	DN vs BC	retro-spective	aortic root procedures	118	3.7	3.0	–	0.851	30 days
Kagan (2021)	DN vs BC	retro-spective	CABG	350	3.8	4.6	–	0.898	in-hospital
Lama (2021)	DN vs BC	RCT	CABG	90	0	2.2	–	1.000	in-hospital
Lenoir (2021)	DN vs BC	PM	aortic root procedures	110	3.6	0	–	0.15	30 days or in-hospital
Reidy (2021)	DN vs BC	retro-spective	CABG in veterans	155	0	2.4	–	0.5	30 days
Ross (2021)	DN vs BC	retro-spective	all cardiac procedures	497	1	2	–	0.43	in-hospital
Sanri (2021)	DN vs BC	retro-spective	CABG	255	5.3	5.7	–	0.892	30 days
Sevuk (2021)	DN vs BC	PM	all cardiac procedures and XC time > 90 min	176	2.3	3.4	–	1.000	in-hospital
Algarni (2020)	DN vs BC	retro-spective	all cardiac procedures	305	0.4	1.4	–	0.43	in-hospital
Chen (2020)	DN vs BC	PM	acute aortic dissection	122	4.83	6.67	–	0.964	in-hospital
George (2020)	DN vs BC	PM	CABG or valve	286	0	0	–	–	30 days
Kirisci (2020)	DN vs BC	RCT	CABG	60	6.67	3.33	–	0.55	in-hospital
Kuserli (2020)	DN vs BC	PM	aortic root procedures	72	2.8	2.8	–	1.000	in-hospital
Schutz (2020)	DN vs BC	PM	CABG or CABG and valve	670	1.8	2.1	–	0.758	operative mortality
Shi (2020)	DN vs HTK	PM	aortic root procedures	86	0	0	–	–	in-hospital

Table 7. continuation

Article	Cardio- plegia	Study design	Cardiac procedures	No. of pa- tients	RBC transfusions			p value	info
					DN	BC	HTK		
Ucak (2020)	DN vs HTK	retro- spective	AVR	137	0	1.2	–	0.512	30 days
Willekes (2020)	DN vs HTK	PM	aortic root procedures	344	5.2	4.7	–	0.8035	30 days
Kuciński (2019)	DN vs BC	retro- spective	all cardiac procedures	2108	3.6	3.0	–	0.54	in-hospital
Luo (2019)	DN vs HTK	PM	MI AVR/ MVR/DVR	132	1.52	1.52	–	1.000	in-hospital
Pragliola (2019)	DN vs BC	PM	all cardiac procedures	204	3.9	3.9	–	1.000	in-hospital
Sanetra (2019)	DN vs BC	RCT	AVR	150	1.3	0	–	1.000	30 days
Timek (2019)	DN vs BC	PM	CABG	650	1.5	1.2	–	1.000	30 days
Kim (2018)	DN vs HTK	PM	valve procedures	208	0	1.9	–	> 0.99	30 days or in-ho- spital
Koeckert (2018)	DN vs HTK	PM	MI AVR	118	0	0	–	–	30 days
Willekes (2020)	DN vs HTK	retro- spective	all cardiac procedures	2108	3.6	3.0	–	0.54	in-hospital
Kuciński (2019)	DN vs HTK	retro- spective	all cardiac procedures	2108	3.6	3.0	–	0.54	in-hospital
Luo (2019)	DN vs BC	PM	MI AVR/ MVR/DVR	132	1.52	1.52	–	1.000	in-hospital
Pragliola (2019)	DN vs BC	PM	all cardiac procedures	204	3.9	3.9	–	1.000	in-hospital
Sanetra (2019)	DN vs BC	RCT	AVR	150	1.3	0	–	1.000	30 days
Timek (2019)	DN vs BC	PM	CABG	650	1.5	1.2	–	1.000	30 days
Kim (2018)	DN vs BC	PM	valve procedures	208	0	1.9	–	> 0.99	30 days or in-hospital
Koeckert (2018)	DN vs BC	PM	MI AVR	118	0	0	–	–	30 days
Sabik (2018)	DN vs BC	PM	MI MVP	124	0	0	–	–	in-hospital
Ad (2017)	DN vs BC	RCT	CABG and/or single valve	89	0	0	–	–	operative mortality
Hamad (2017)	DN vs BC	retro- spective	AVR + CABG	50	0	4	–	1.000	in-hospital
Ziazadeh (2017)	DN vs BC	PM	MI AVR	126	0	3.1	–	ns	30 days
Kim (2016)	DN vs BC	PM	all cardiac procedures	78	2.6	0	–	–	operative mortality
Mischra (2016)	DN vs BC	retro- spective	CABG and DVR	100	0	2	–	0.314	in-hospital

Table 7. continuation

Article	Cardio-plegia	Study design	Cardiac procedures	No. of patients	RBC transfusions			p value	info
					DN	BC	HTK		
Vistarini (2016)	DN vs BC	retro-spective	MI AVR	46	0	0	-	> 0.99	in-hospital
Ota (2015)	DN vs BC	PM	AVR	108	0	0	-	-	in-hospital
Ramanathan (2015)	DN vs BC	retro-spective	all cardiac procedures	142	7.0	1.4	-	ns	in-hospital
Sorabella (2014)	DN vs BC	retro-spective	reoperative AVR	113	0	1.6	-	1.000	in-hospital
Duan (2021)	DN vs HTK	PM	complex valve surgery	146	4.1	-	8.2	0.302	30 days
Gunaydin (2020)	DN vs HTK	RCT	MI AVR	200	2.10	-	1.04	0.7	in-hospital
Lee (2020)	DN vs HTK	PM	MICS	456	0.4	-	0.4	> 0.99	30 days or during index hospitalization

risk of reoperation caused by bleeding was higher in the DNC group. In the sub-analysis of CABG cases the post-operative levels of creatinine were significantly lower in the DNC group but so were the pre-operative levels. A RCT by Santera et al. [26] reported that the rate of significant creatinine increase (> 25% or > 0.5mg/dL) or AKI was greater in the CBC group. The retrospective study by Eris et al. [18] also showed that AKI was more frequent in the CBC group. Further analysis revealed it only applies to the AKI Stage 1. There were no differences in the rate of other complications (cerebrovascular incident, infection, AF, bleeding, respiratory failure) or mortality. Stage 1 AKI (or any AKI) in CBC was also more frequent in a retrospective study by Kagan et al. [21] In logistic regression analysis AKI was correlated, among others, with increased cardioplegia volume.

Luo et al. [33] showed significantly lower levels of blood urea nitrogen (BUN) in the DNC group, without differences in the post-operative levels of creatinine, eGFR or pre-operative BUN. However, the DNC group had a significantly lower Euro SCORE II. Schutz et al. [37] reported a significantly higher rate of neurological complications in the postoperative period in DNC for the overall cohort (CABG + valve procedure), as well as for the subgroup with LVEF < 35%. The rate of stroke was also significantly higher in this subgroup when DNC was used. The frequency of total infections and total sternal infections was greater in the CBC group for the overall cohort. Overall, most studies did not reveal differences in the rate of

postoperative complications between groups. It is noteworthy that almost all of the reviewed articles analyzed different kinds of complications with different definitions. Early mortality was the complication that was most often studied (in 37 studies, see Table 7) [1-6, 8-21, 23-26, 28-35, 37, 39-42].

Echocardiographic findings

Only George et al. [39] reported significantly lower LVEF measured on the 5th post-operative day (POD) in the DNC group, although the median values in both groups were in the normal range.

Four [1, 6, 16, 36] studies (including one RCT), all describing different types of cardiac surgeries, showed better echocardiographic parameters in the DNC group measured at different time points during hospitalization. No differences were shown in other studies comparing DNC and CBC (Table 8).

Two RCTs on DNC versus HTK [22, 25] reported no differences in the LVEF measured before discharge. In the study by Mehrebenian et al. [22], LVEF measured in the OR after weaning from CPB was also comparable. In Duan et al. [23] LVEF measured in the postoperative period was also similar between groups, however systolic volume and cardiac output (as echocardiographic parameters) were significantly lower in HTK, both in the general cohort and subgroup of XC ≥ 120 minutes. Whereas the sub-analysis of cases with XC < 120 minutes showed no differences. [23]

Length of ICU and hospital stay, mechanical ventilation time

The majority of articles presented comparable hospital and intensive care unit (ICU) length of stay (LOS) (Table 9) and time of mechanical ventilation between the DNC and

CBC cases. A shorter ICU stay for the DNC group was reported in 2 studies [4, 38], with patients undergoing all types of cardiac procedures. In their study concerning AVR reoperations, Sorabella et al. [29] reported no differences in the ICU LOS and mechanical ventilation time, although hospital LOS was significantly shorter when DNC was used. Similar results

Table 8. LVEF

Article	Cardio-plegia	Study design	Cardiac procedures	No. of patients	postoperative LVEF [%]			p value	Time point
					DN	BC	HTK		
Eris (2021)	DN vs BC	retrospective	aortic root procedures	118	50.3±7.5	49.7±8.2	-	0.714	before discharge
Lama (2021)	DN vs BC	RCT	CABG	90	56.33±8.94	50.45±8.55	-	< 0.001	before discharge
Lenoir (2021)	DN vs BC	PM	aortic root procedures	110	54±1	55±1	-	0.3	before discharge
Sevuk (2021)	DN vs BC	PM	all cardiac procedures and XC time > 90 min	176	55(45-55)	50(45-55)	-	0.08	before discharge
Shu (2021)	DN vs BC	PM	all cardiac procedures	326	60.0 (50.5-62.0)	59.0 (48.5-61.0)	-	0.263	within 48h post-op
George (2020)	DN vs BC	PM	CABG or valve	286	53.4 (45.1-57.1)	56.0 (51.7-57.6)	-	< 0.001	5th post-op day
Kuserli (2020)	DN vs BC	PM	aortic root procedures	72	53.75±6.14	50.00±5.07	-	0.006	before discharge
Shi (2020)	DN vs BC	PM	aortic root procedures	86	60.07±9.57	58.37±8.83	-	0.395	post-op
Ucak (2020)	DN vs BC	retrospective	AVR	137	56.70±5.30	55.42±4.90	-	0.508	routine post-op
Pragliola (2019)	DN vs BC	PM	all cardiac procedures	204	46±10	47±9	-	0.5	before discharge
Sanetra (2019)	DN vs BC	RCT	AVR	150	54.64±6.49	54.61±5.63	-	0.708	2-3 days post-op
Timek (2019)	DN vs BC	PM	CABG	650	53±13	54±12	-	0.36	follow-up
Sabik (2018)	DN vs BC	PM	MI MVP	124	56.70±6.92	54.20±8.72	-	0.077	on discharge
Hamad (2017)	DN vs BC	retrospective	AVR + CABG	50	59.4±8.3	58.1±8.9	-	0.601	post-op
Vistarini (2016)	DN vs BC	retrospective	MI AVR	46	61.5±6.0	59.1±3.8	-	0.15	on discharge
Sorabella (2014)	DN vs BC	retrospective	reoperative AVR	113	48.5±10.7	46.8±10.9	-	0.401	post-op
Duan (2021)	DN vs HTK	PM	complex valve surgery	146	57(49-62)	-	54(45-62)	0.261	"perioperative"
Gunaydin (2020)	DN vs HTK	RCT	MI AVR	200	41.0±10.0	-	42.5±10.0	0.6	before discharge
Mehrabanian (2018)	DN vs HTK	RCT	complex cardiac procedures	40	41.67±12.32	-	41.79±7.15	0.97	on discharge

Table 9. Hospital and ICU LOS

Article	Car-dio-plegia	Study design	Cardiac procedures	No. of patients	ICU LOS [d] or [h]			p value	Hospital LOS [d]			p value
					DN	BC	HTK		DN	BC	HTK	
Eris (2021)	DN vs BC	retro-spective	aortic root procedures	118	2.3 ±0.6 [d]	2.4 ±0.9 [d]	-	0.165	7.6±1.4	7.8 ±2.2	-	0.489
Kagan (2021)	DN vs BC	retro-spective	CABG	350	2.94± 3.20 [d]	2.90± 3.30	-	0.494	7.34 ±3.12	7.70 ±4.14	-	< 0.001
Lama (2021)	DN vs BC	RCT	CABG	90	3.47 ±1.09 [d]	3.49± 1.75 [d]	-	0.35	6.07 ±1.34	6.64 ±2.81	-	0.21
Lenoir (2021)	DN vs BC	PM	aortic root procedures	110	1.80± 0.20 [d]	1.60± 0.15 [d]	-	0.32	5.5±0.3	5.6±0.3	-	0.27
Reidy (2021)	DN vs BC	retro-spective	CABG in veterans	155	3.9 (2.9- 5.9) [d]	3.5 (2.1-5) [d]	-	0.16	7.4 (6.1- 13.2)	7.2 (6-11.1)	-	0.28
Ross (2021)	DN vs BC	retro-spective	all cardiac procedures	497	47 (25-91) [h]	47 (26-114) [h]	-	0.27	8 (6-12)	7 (6-10)	-	0.87
Sanri (2021)	DN vs BC	retro-spective	CABG	255	3.06± 3.90 [d]	3.08± 3.31 [d]	-	0.52	6.89 ±3.27	7.23± 2.56	-	< 0.001
Sevuk (2021)	DN vs BC	PM	all cardiac procedures and XC time > 90 min	176	1 (1-2) [d]	1 (1-2) [d]	-	0.09	5 (5-6)	5 (5-6)	-	0.24
Shu (2021)	DN vs BC	PM	all cardiac procedures	326	1.97± 1.58 [d]	2.55± 2.36 [d]	-	0.0103	-	-	-	-
Algarni (2020)	DN vs BC	retro-spective	all cardiac procedures	305	3 (2-5) [d]	4 (2-6) [d]	-	0.047	9 (7-14)	11 (7-19)	-	0.1
Chen (2020)	DN vs BC	PM	acute aortic dissection	122	7.9± 2.1 [d]	8.1 ±2.3 [d]	-	0.617	-	-	-	-
George (2020)	DN vs BC	PM	CABG or valve	286	3.1±0.8 [d]	3.1±0.5 [d]	-	0.931	7 (6-8)	7 (6-9)	-	ns
Kirisci (2020)	DN vs BC	RCT	CABG	60	2.23± 0.68 [d]	2.23± 0.43 [d]	-	1.000	7.11 ±0.63	7.00 ±0.64	-	0.52
Kuserli (2020)	DN vs BC	PM	aortic root procedures	72	2 (2-5) [d]	3 (2-6) [d]	-	0.681	-	-	-	-
Sharma (2020)	DN vs BC	retro-spective	AVR + MVR	209	2.10± 0.34 [d]	2.10± 0.30 [d]	-	0.447	5.90 ±0.90	5.98 ±0.90	-	0.52
Shi (2020)	DN vs BC	PM	aortic root procedures	86	52.19 ±75.10 [h]	34.74 ±27.41 [h]	-	0.156	8.88 ±3.93	8.93 ±3.63	-	0.955
Ucak (2020)	DN vs BC	retro-spective	AVR	137	29.6 ±5.1 [h]	28.4 ±6.6[h]	-	0.685	6.2±2.8	6.2±2.4	-	0.795
Koeckert (2018)	DN vs BC	PM	MI AVR	118	43.1± 57.8 [h]	29.3± 23.9 [h]	-	0.098	6.7±4.5	5.4±2.3	-	0.116
Hamad (2017)	DN vs BC	retro-spective	AVR + CABG	50	59.4± 42.8 [h]	44.3± 41.0 [h]	-	0.213	6.8±2.5	8.7±5.4	-	0.127
Ziazadeh (2017)	DN vs BC	PM	MI AVR	126	33±43[h]	34±50 [h]	-	ns	5.8±2.5	5.9±2.7	-	ns
Vistarini (2016)	DN vs BC	retro-spective	MI AVR	46	51.7 ±52.9 [h]	51.6 ±50.4 [h]	-	> 0.99	7.9±3.8	7.1±2.3	-	0.38
Ota (2015)	DN vs BC	PM	AVR	108	2.6 ±2.1 [d]	3.7 ±3.9 [d]	-	0.079	8.3 ±10.6	8.8±8.1	-	0.816
Rama-nathan (2015)	DN vs BC	retro-spective	all cardiac procedures	142	-	-	-	-	14.0	16.2	-	0.3135

Table 9. continuation

Article	Cardio-plegia	Study design	Cardiac procedures	No. of patients	ICU LOS [d] or [h]			p value	Hospital LOS [d]			p value
					DN	BC	HTK		DN	BC	HTK	
Sorabella (2014)	DN vs BC	retrospective	reoperative AVR	113	2.8 ±2.0 [d]	4.1±7.0 [d]	-	0.193	7.9±3.4	10.1 ±7.2	-	0.035
Duan (2021)	DN vs HTK	PM	complex valve surgery	146	29 (20-68) [h]	-	43 (21-75) [h]	0.024	8 (7-11)	-	9 (7-11)	0.933
Gunaydin (2020)	DN vs HTK	RCT	MI AVR	200	1.8 (0.5-3.6) [d]	-	1.0 (0.4-1.7) [d]	0.41	6.4 (4.5-8.4)	-	5.6 (4.9-7.1)	0.56
Lee (2020)	DN vs HTK	PM	MICS	456	1 (1-2) [d]	-	1 (1-2) [d]	0.468	7 (6-10)	-	7 (6-9)	0.126
Mehrabanian (2018)	DN vs HTK	RCT	complex cardiac procedures	40	2.22 ±0.45 [d]	-	2.53 ±0.68 [d]	0.101	-	-	-	-

can be found in two retrospective studies on isolated CABG [20-21]. Significantly shorter mechanical ventilation time in the CBC group was reported by Vistarini et al. [34]. In a sub-analysis of cases with longer XC time requiring a second DNC or HTK dose, Gunaydin et al. [25] reported significantly longer ICU LOS and hospital LOS in the DNC group. No differences were shown when only one dose of either DNC or HTK was used. Mechanical ventilation time was comparable in all analyses. Duan et al. [23] observed that the ICU LOS was significantly shorter in the DNC group, both in the general cohort after PSM and in the sub-analysis of cases with XC < 120 minutes. In XC ≥ 120 minutes cases ICU LOS was comparable between groups. None of the sub-analyses in that study revealed differences in the hospital LOS.

Discussion

All of the currently used cardioplegic solutions are safe and well-designed. The oldest of them, high-potassium CBC, is also the most widely used in the world [43]. CBC has great buffering properties and ensures cardiac arrest in a highly oxygenated environment. Its oncotic properties prevent cell oedema and its twenty minutes re-dosing period provides re-oxygenation and washout of waste products, which improves the preservation of ATP [39]. DNC developed by the team from Boston Children's Hospital [44] is also a high-potassium solution. The main purpose of its chemical composition was to ensure the lowest possible intracellular accumulation of calcium, which is particularly elevated in depolarizing cardiac arrest. Depolarization creates higher sodium and calcium gradient, which leads to cell calcium overload – the main factor of IRI. Calcium influx is one of the mechanisms leading to mitochondrial dysfunction and reperfusion fibrillation [23].

High potassium levels may also lead to vasoconstriction and disrupt the endothelium function in coronary and peripheral vessels aggravating the damage during reperfusion [45]. DNC contains lidocaine (a Na⁺-K⁺ pump blocker) which counteracts the unfavourable effects of depolarization by stabilizing the cell membrane, lowering excitability and elongating the refraction period [46]. All these actions reduce the probability of hypercontraction due to high Ca²⁺ concentration. What is more DNC itself contains a small amount of Ca²⁺. Additionally, lidocaine has a protective effect on the endothelium.

In some cardioplegia strategies, lidocaine can be administered into the peripheral circulation [31], however, it is more effective when injected directly into the coronary circulation. Unlike CBC, DNC has low nutritional value due to the small amount of blood and glucose. On the other hand, a small amount of blood leads to lower hematocrit (ca. 6-7%), which compared to CBC (ca. 26-32%) may provide better distribution in the coronary microcirculation. Such lower hematocrit could be beneficial, particularly in low temperatures when blood viscosity increases.

Better distribution means better cardioprotection and lower postoperative cardiac enzymes. HTK acts differently from the two above-mentioned solutions. A low-sodium solution causes membrane hyperpolarization, which leads to a reversible diastolic arrest. Better cell membrane stabilisation provides lower permeability for ions such as Ca²⁺. An additive of Mg²⁺ in HTK also stabilizes cardiomyocyte cell membranes, yet lidocaine seems to be superior and more effective.

Quality of cardioprotection: cardiac enzymes

Cardiac enzymes are one of the most reliable markers of cardioprotection during the cardiac surgery procedure. Elevated postoperative Tn or CK-MB level correlates with early

outcomes and mortality of cardiac surgery patients. The majority of analyzed studies showed equally good or better cardioprotection in DNC cases when correlated with cardiac enzyme levels. The weak point is again the heterogeneity of reporting. Study protocols differ in terms of the time point of collecting blood samples, the measurement's accuracy over time [47], application and degree of hypothermia, cardioplegia administration and redosing protocols.

Quality of cardioprotection: post cross-clamp ventricular fibrillation

An indirect sign of cardioprotection is the heart's electrical activity during the procedure. In two studies analyzing this parameter, DNC was more effective in lowering the electrical activity of the heart.

Spontaneous return of SR after cross-clamp removal also gives information about cardioprotection. Longer ischemia and lower quality of cardioprotection increase the probability of VF and the need for defibrillation. Once again, the reported values are not homogeneous but are enough to make some conclusions. What draws attention is a significantly higher percentage of spontaneous SR return in DNC groups in the vast majority of the analysed studies. This illustrates good cardioprotection and control of Ca^{2+} accumulation mechanisms in cardiomyocytes, which is a main factor of VF after XC [42].

Hypothermia prolongs repolarization and is a known pro-arrhythmic factor. Constant cooling of the myocardium during many CBC repetitions may trigger VF. Slow rewarming of the heart after only one dose of DNC could be a protective factor [26]. However, this effect is not visible in HTK (also a single-dose cardioplegia solution) and probably plays an additional role in cardioplegia composition.

Less VF after XC removal means less direct current shocks and less myocardial damage, which may lower the concentrations of post-operative cardiac enzymes. Discrepancies in the VF:SR ratio noted in the studies may be due to the differences in the cardioplegia administration protocols or the patient population.

Quality of cardioprotection: echocardiographic parameters

Decreased contractility in the post-operative period may be the result of poor cardioprotection. All but one study [39] showed similar or better cardiac function in echocardiographic examination in the DNC groups when compared with CBC or HTK. It is noteworthy that not all of the articles analyzed changes between pre- and post-operative examinations. Duan et al. [23] comparing DNC and HTK reported better post-operative systolic volume and cardiac output measured by echocardiography in the former. However, this ratio does not necessarily indicate better heart

function, only an increase in extracellular fluid. There is no evidence that LV mass influences cardioprotection [12]. However, LV hypertrophy and the presence of coronary disease in adults may have a negative effect the quality of cardioprotection in comparison with the pediatric population. Hypertrophied myocardium has a metabolism similar to that of fetal myocardium (increased glycolysis, reduced oxidation of fatty acids), which leads to reduced ischemia tolerance. This may suggest a better effect of DNC developed for similar conditions.

Postoperative complications

There is no evidence that the use of DNC reduces the number of postoperative complications. A relatively small number of analyzed cases may cause problems with obtaining statistical significance of rare complications. Differences in reporting also make it difficult to draw clear conclusions, as in the case of vasoactive or inotropic agent administration. LCOS may be a consequence of inadequate cardioprotection requiring vasoactive/inotropic support or even mechanical circulatory support. Almost every study had a different method of reporting data, thus making it difficult to compare them. However, in general patients with DNC had a similar or even lower need for vasoactive and inotropic agents.

The possible indirect influence of DNC on renal function is noteworthy. In four studies the DNC patients had better renal parameters [26, 33] or less frequent occurrence of AKI [18, 21, 26]. In studies by Eris and Kagan, this only applied to stage 1 AKI [18, 21]. Sanetra et al. hypothesized that lidocaine as a vasodilator could improve renal flow which in addition to shorter CPB and better cardiac function in the postoperative period may have a positive effect on kidney function [26]. A trend towards better renal function is also seen in metanalysis by Misra et al. [48].

Questions and limitations of the review

Two main DNC dosing protocols can be found in the literature. The first method is a constant volume (usually 1000 or 1500 ml) delivered regardless of the patient's body weight. The second approach is dosing based on the patient's body weight (typically 20 mL/kg), with or without an indication of the maximum dose.

However, the method of administering the additional DNC doses remains unclear due to the lack of a standard dosing schedule. The most common rule is re-dosing $\frac{1}{2}$ of the initial DNC dose after 60 minutes of XC if the expected total XC time is > 90 minutes. Many modifications to the re-dosing period or the volume and composition of the second DNC dose can be found in the literature [49-52]. The authors of the analyzed studies did not always include the protocols for administration and re-dosing of DNC. Those potential differences in dosing may be responsible for inconsistent results,

particularly because the vast majority of the reviewed articles were retrospective.

Conclusions

The currently used cardioplegic solutions are safe and have proven properties to protect the heart against the effects of ischemia during open-heart surgery. The differences between DNC, CBC and HTK described in the analyzed studies do not disqualify any of the solutions but may suggest the need for further research, particularly in the context of longer XC times. More randomized trials are needed, possibly also examining cardiac function in a long-term follow-up.

Acknowledgements

None.

Funding

None.

Conflicts of interest

The authors do not have any conflict of interest in this project.

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A quantitative analytical investigation on the oxycodone side effects listed in recent clinical trials

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Abstract

Background: Oxycodone is a semi-synthetic narcotic analgesic medication with numerous side effects. Patients who use oxycodone may have one or more side effects depending on their clinical status and/or other factors. Our aim was to analyze clinical trials for any potential pattern related to the side effects of oxycodone. **Methods:** We searched the ClinicalTrials.gov database for clinical studies conducted around the world regarding oxycodone. A number of general data analytic and text manipulation techniques were used in the Python programming language. **Results:** We analyzed 842 clinical trials involving oxycodone. Our results suggest that the researchers focused on oxycodone's typical side effects (e.g. vomiting), as opposed to the less evident and/or long-term side effects e.g. depression, confusion and constipation. **Conclusions:** The adverse effects of oxycodone most commonly reported in the clinical trials are nausea, vomiting and depression. Despite the clinical use of oxycodone in all the age groups (including infants), very few of the publicly-available clinical trials included participants < 18 years of age.

Keywords: oxycodone • nausea • vomiting • itching • confusion

Citation

Toussi AG, Toosi FG. A quantitative analytical investigation on the oxycodone side effects listed in recent clinical trials. Eur J Transl Clin Med. 2023;6(1):58-63.

DOI: [10.31373/ejtcmed/163415](https://doi.org/10.31373/ejtcmed/163415)

Introduction

Strong analgesics are generally divided into two groups known as opiates and *opioids*. The first group, *opiates*, include opium, morphine, codeine, and heroin which are derived from naturally-occurring plants [1]. In contrast,

opioids such as dextromethorphan, methadone, dextro-propoxyphene, oxycodone and tramadol are fully synthesized in the laboratory. One of the main reasons for using oxycodone is to tranquilize moderate to severe pain such as diabetic foot, cancer-related pains, osteoarthritis, low back pain, gastrointestinal pain, etc [2].

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Available online: www.ejtcmed.gumed.edu.pl

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Oxycodone has several pharmaceutical forms such as elixir, vials, and capsules. Oxycodone acts directly on the opioid receptors, namely μ (μ), κ (κ) and δ (δ), in the central nervous system and provides pain relief by interrupting the pain signal transduction between the brain and the body [3-4]. Although this opioid often can relieve moderate to severe pain, it also has some hazardous outcomes, therefore it should be prescribed only when non-opioids are not effective.

While the mass media raise alarm about an “opioid epidemic” in the United States, the data confirms that number of prescriptions for opioids in the United States increased by 35.2% from 2000 to 2009 [5]. The same article also reported that the dosage of opioids (in milligrams) increased by at least 100% in US pharmacies during the period of 2000 to 2010. This huge increase in opioid use, including oxycodone, also means that the adverse effects of these medications are more frequent. Some of the common adverse effects of oxycodone include dizziness, confusion, constipation, diarrhea, vomiting, and nausea. Oxycodone toxicity is increasing nowadays because of deliberate and/or accidental use. Therefore, oxycodone should be prescribed carefully by a physician who knows how to manage its adverse effects. To this end, there is a need to design and run new experiments to study opioid side effects. The aim of this study was to assess recent clinical trials for any potential pattern related to the side effects of oxycodone.

Materials and Methods

ClinicalTrials.gov (<https://clinicaltrials.gov/ct2/home>) is a database of privately and publicly funded clinical studies conducted around the world. It collects data about several features of these studies, including inclusion and exclusion criteria, age range, background history, patient status, etc. Details of the studies (clinical trials) are entered manually. Some of the data in this database is labelled/categorized

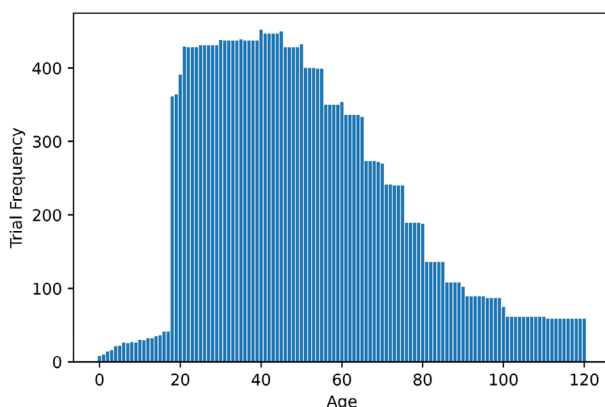


Figure 1. Age of oxycodone clinical trial participants

data and some (e.g. study title or outcome measures) is open-format data (e.g. text) without control label on terminology and structure.

We searched this database for all clinical trials that contained the term ‘oxycodone’ and retrieved a total of 842 records. The status of these clinical trials ranged from ‘recruiting’ or ‘active’ to ‘completed.’ The retrieved number of trials was not large enough to use more advanced tools such as artificial intelligence (i.e. machine learning), we relied on general analytic techniques (such as data cleansing, data categorization and textual manipulation) in the Python 3.7 programming language (Python Software Foundation, Delaware, USA) and its modules e.g. Numpy, Pandas and Matplotlib.

Results

The results of each analysis are presented separately in this section.

Inclusion Criteria/Age

As shown in Figure 1, the most common age group among the participants of the oxycodone-related clinical trials is 20 to 45 years. Hardly any trials included participants who were < 20 years of age. We noted 2 trials involving infants. Morse et al. indicated that oxycodone is prescribed for patients as young as premature neonates as well as adults of any age, therefore, it is important to investigate the side effects of such medication for a wider age-range i.e., neonates to adolescent [6].

Adverse Effects of Oxycodone

Next, we analyzed oxycodone’s adverse effects described in these clinical trials. We focused on the 6 most common adverse effects of oxycodone as described by Gallego et al. (see Table 1), itching and depression [7-8]. Although itching and pruritus are synonymous, authors used either term in their studies, therefore we used both terms in order to find all studies with this particular adverse effect.

These data were manually entered into the ClinicalTrials.gov database, thus there might be inconsistent terminology describing the same condition, e.g. vomit and vomiting. Therefore, text analytical functions were applied to extract every variation.

We looked at the rate at which the study title and, more importantly, the outcome measures included either of these adverse effects described above. As shown in Figure 2, nausea, vomiting and depression were most often mentioned in the trials’ outcome measures. Whereas in the study titles adverse effects were much less frequently mentioned and constipation leads the list. Study titles are much more exposed online, outside the ClinicalTrials.gov database as well,

therefore one can conclude that constipation has been one of the main concerns of the researchers [9].

Next, we assessed the age distribution of patients reporting each of these adverse effects. Majority of the patients reporting the three adverse effects of oxycodone most commonly noted in the clinical trial outcome measures (nausea, vomiting, depression) all have a very similar age distribution: 20-60 years of age, with a decreasing number of studies involving an older age group (Fig. 3 A-C). Constipation is another adverse effect that is prominent in the 40-60 years of age group, with no participants younger than 20 (Fig. 3 D). This lack of attention to the younger age group in the clinical trials surprised us given how common is constipation secondary to oxycodone [10]. Pruritus and itching (see Fig. 3 E and G) have a similar distribution, albeit slightly younger. Confusion was mentioned in only one trial with participants > 20 years of age (Fig. 3 D). Dizziness has been the focus of around 25 studies for the age range of 20-40 and 1 or 2 studies for the older age group (Fig. 3 F). Finally, drowsiness was discussed in only 2 studies with the participants' age range of 20-50.

Discussion

As mentioned in the Results section, most of the analyzed trials focused on three adverse effects: nausea, vomiting and depression. However, since nausea and vomiting may be referring to similar medical state, we combined them into one category and reproduced the figure.

As shown in Figure 4, nausea and vomiting have the highest attention of researchers, while other side effects that might not be immediately evident received less atten-

Table 1. The common side effects of oxycodone reported by Gallego et al.

Side Effects	Likelihood
Constipation	25-30%
Nausea	25%
Drowsiness	25%
Dizziness	15%
Vomiting	10-15%
Pruritus	10-15%

tion. This might be due to the difficulty of keeping patients on track for a long time which requires further investigation in future works. On the other hand, since several studies focused on vomiting and nausea, one can argue that these side effects are more frequent than others, therefore, solutions to reduce these side effects may be worth investigating. Basic solutions include reducing physical movement immediately after eating and consuming enough fiber for the proper movements of the digestive system after oxycodone use. It is also worth comparing the nausea effects of oxycodone with other opioid-related medications such as methadone and oxycodone first as a pilot study in animals such as guinea pigs [11-12] and then in humans if the preliminary results are promising.

Another highlight of this study is the lack of attention to the younger age range. Studies [13-15] show that the use of oxycodone is also common in the younger age group (< 20 years of age) while our findings show that this age range was neglected (or less focused) in recent clinical trials. The lack of such important details is a limitation in treating youngsters with opioids.

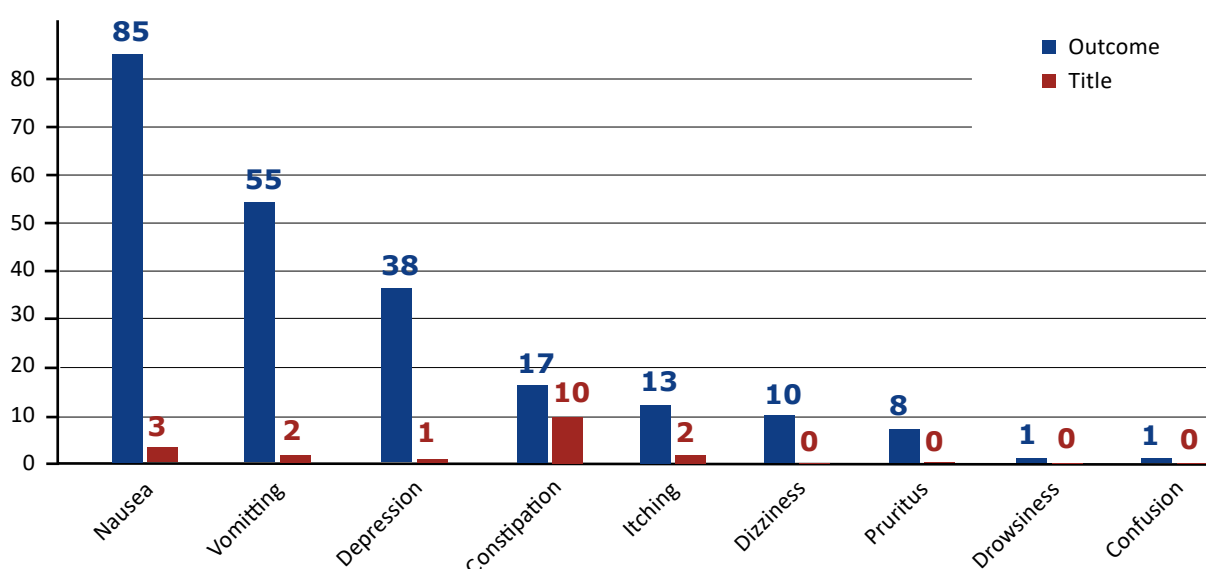


Figure 2. Number of trials that contained the particular adverse effect in the study title or outcomes

Limitations

The main limitation of our analysis is related to the dataset we obtained. Although researchers usually publish their results and methodologies, this is not mandatory therefore there might be relevant trials that were not included in the ClinicalTrials.gov database. In addition, this database includes only those studies whose results have been made to some extent public.

Conclusions

The adverse effects of oxycodone most commonly reported in the clinical trials are nausea, vomiting and depression. Despite the clinical use of oxycodone in all the age groups (including infants), very few of the publicly-available clinical trials included participants < 18 years of age.

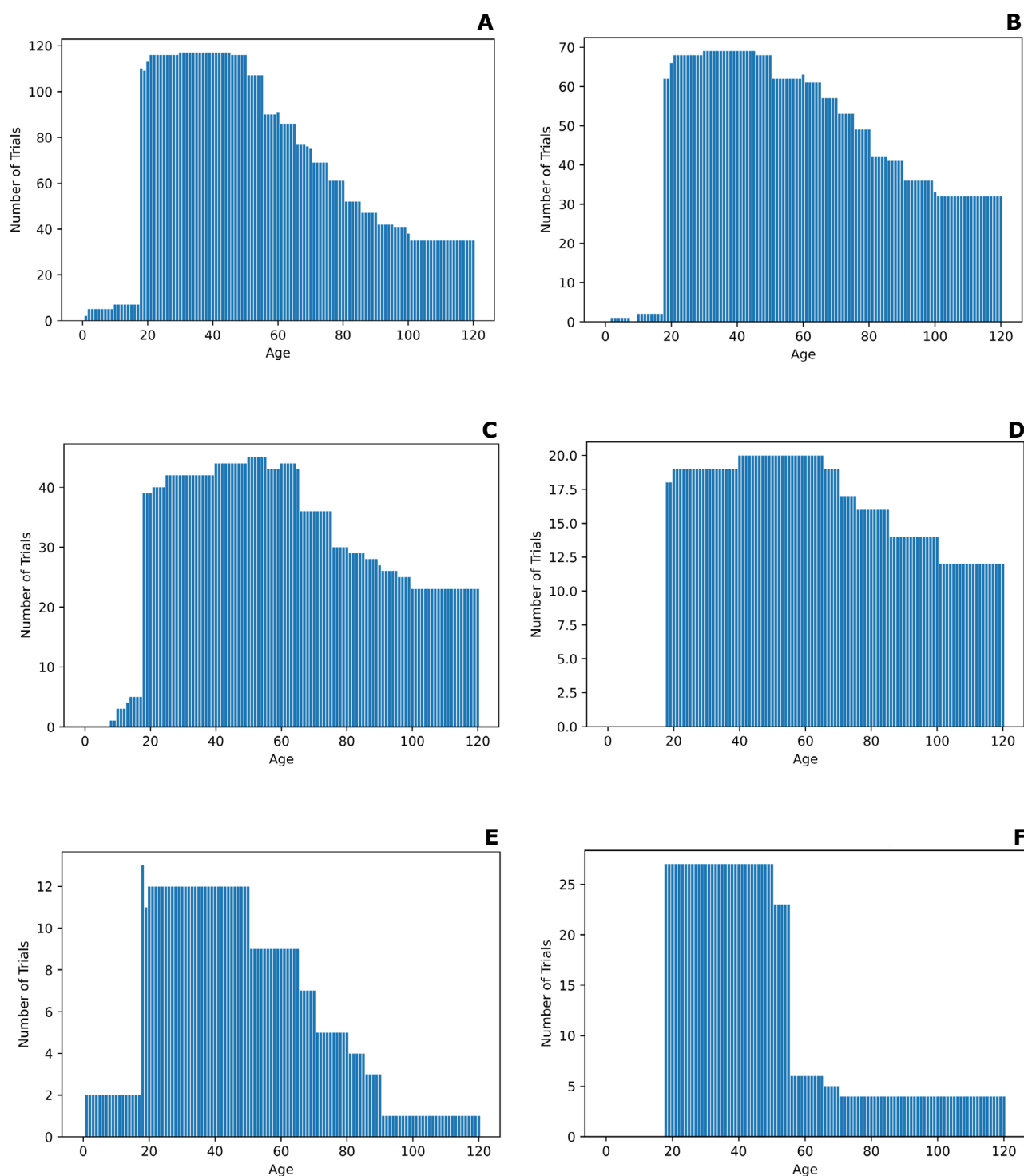
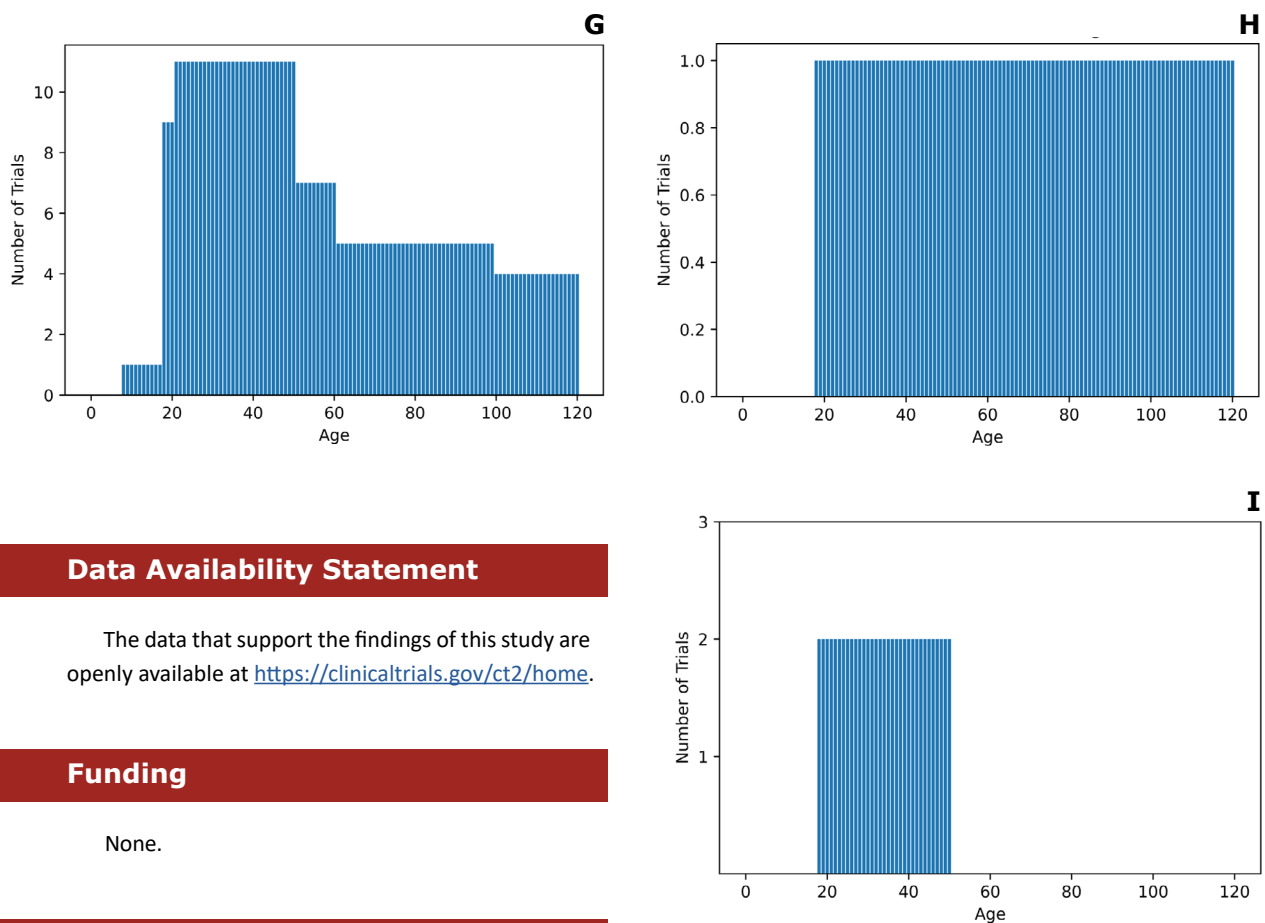


Figure 3. The details of the age distribution of clinical trial patients reporting the following adverse effects: A – nausea, B – vomiting, C – depression, D – constipation, E – itching, F – dizziness



Data Availability Statement

The data that support the findings of this study are openly available at <https://clinicaltrials.gov/ct2/home>.

Funding

None.

Conflicts of interests

None.

Figure 3. The details of the age distribution of clinical trial patients reporting the following adverse effects: G – pruritus, H – confusion, I – drowsiness

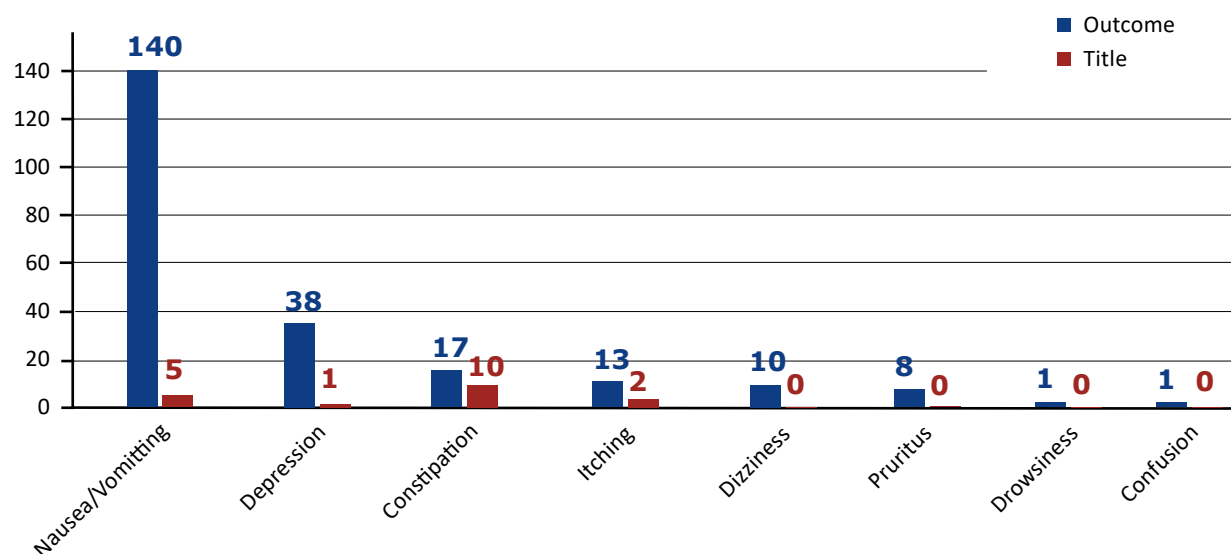


Figure 4. Number of Trials that includes each side-effect separated by Outcome and Title details and ordering by outcome details

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Giant cell tumor of tendon sheath in the pediatric population – review

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Abstract

Giant cell tumor of tendon sheath is a rare and benign soft-tissue tumor, which is even less common in the pediatric population. It may be connected with chromosomal translocations, often the 1p11-13. Symptoms include pain, swelling, palpable mass or limited range of motion. Magnetic resonance is the diagnostic method of choice. Histopathological image obtained from biopsy should be also evaluated. The gold standard of treatment is total surgical resection of the tumor, as incomplete excision may result in recurrence. Physicians should also consider the future function of the affected joint or limb. Our aim was to review the available literature about GCTTS in the pediatric population.

Keywords: orthopedics · pediatric oncology · giant cell tumor of tendon sheath

Citation

Kopcik K, Koberling A, Koper J, Bichalska-Lach M, Rudzki M, Waniczek D. Giant cell tumor of tendon sheath in the pediatric population – review. Eur J Transl Clin Med. 2023;6(1):64-69.

DOI: [10.31373/ejtcmm/162882](https://doi.org/10.31373/ejtcmm/162882)

Introduction

Giant cell tumor of tendon sheath (GCTTS), also known as tenosynovial giant cell tumor, is the most frequent form of giant cell tumors and the second most common soft tissue tumor of the hand (after ganglion cyst) [1-3]. GCTTS is

most often localized in the hands [4]. This tumor originates from the sheath, bursa or joints [2, 5-6]. Tumor is typically benign, but with aggressive or malignant potential [2, 4]. GCTTS may occur as localized or diffuse form [7-8] and is most common among adults 30 to 50 years of age. [2, 4, 7, 9-10]. The global incidence rate of GCTTS located in the digits, limb and

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the diffuse-type are respectively 29, 10, and 4 per million person-years [11]. GCTTS is even less common in the pediatric population, with an incidence rate of 2.42 and 1.09 per million person-years in case of localized and diffuse tumors, respectively [12]. The knowledge about pediatric GCTTS is scattered across case reports and case series [7, 13]. Our aim was to review the available literature about GCTTS in the pediatric population.

Material and methods

For the purpose of this narrative review, we searched the PubMed database for articles in English and Polish language regarding GCTTS. The keywords “giant cell tumor of tendon sheath” or “giant cell tumor of tendon sheath pediatric” were used. We focused on articles published in the years 2016 to 2023. Evaluation was based on articles’ titles and abstracts. Main criterion of including the papers to the review was if they concerned pediatric population. After evaluation of abstracts, full-text articles were analyzed, including the references cited.

Results

We found a total of 37 articles, including review articles, case series and case reports from the field of oncology, orthopaedics and radiology. After evaluation, 13 articles were included in our review. The rest of the articles were about adult patients. Cases reports are briefly summarized in the “Pediatric cases of GCTTS” section of this article.

Pathology

GCTTS is mostly located in the hand. Interphalangeal joints are often affected [13]. It may also be found in foot (with tendency to be spotted in forefoot), ankle, knee, hip, elbow or shoulder, although location in large joints or the spine is rare [2, 4]. This tumor can be divided into two types: intra-articular or extra-articular [7]. GCTTS may be described as localized tumor, which tends to be a benign form, and diffuse form, which appears to be more aggressive and more likely to localize in large joints [4]. Local and diffuse forms are morphologically indistinguishable, however location near large joints or local aggressiveness are more characteristic for the diffuse form [13]. The origin of GCTTS is uncertain and the hypothesised pathomechanism involves an inflammatory reaction followed by regenerative hyperplasia or local lipid metabolism abnormality [1, 7]. Recent studies revealed that there may be a connection with giant cell tumors and chromosomal translocations involving short arms of chromosome 1, which are present in most cases of GCTTS [1, 13]. These rearrangements often involve 1p11-13, t(1;2)(p11;q35-36)

or translocations of 16q24 [13-14]. It is noteworthy that 1p13 is a location of CSF1 – Colony Stimulating Factor 1. COL6A3 – collagen 6A3 promoter element is located at 2q37 [8, 14]. Regular CSF1 function is stimulating secretion of macrophage colonies. These translocations result in prolonged lifetime of the CSF1 mRNA and its local over-expression, leading to an inflammatory infiltration of mononuclear and multinuclear giant-cell osteoclasts and macrophages [10, 14-15]. This situation, known as „the landscape effect” leads to the fact that only stromal cells present neoplastic mutation, and infiltration of osteoclasts is a reactive element [14]. Therefore GCTTS can be described as mixed disease: neoplastic and inflammatory [16].

Macroscopically, GCTTS is usually a 1-3.5 cm tumor with fibrous capsule [17]. In some cases, these tumors may present different colors – grey, yellow-orange, brown-ish – as a result of different composition and amount of hemosiderin, fibrin, collagen and histiocytes inside [16, 18].

Microscopic images of GCTTS show macrophage-like cells, which can be described as spindle or polyhedral, synovial cells and osteoblastic multinucleated giant cells. Synovial cells may appear as proliferating. Cells may contain hemosiderin deposits [4, 17]. Some cases may contain xanthoma cells, hyalinization or inflammation factors, ferruginous phagocytes and collagen matrix [7, 16-17]. According to the literature, only 2-16% of cells present neoplastic transformation, and the majority appears as reactive, but not transformed cells [14]. Tumors may appear as lobulated, surrounded by collagen [7]. Malignant forms present tumor necrosis, infiltrative pattern, prominent nucleoli and high nuclear to cytoplasmic ratio. Shape is round to oval. Cells are mostly mononuclear, and may contain up to 5 mitoses per 10 high power fields. It is vital to take under consideration the fact that both malignant and benign cells contain apoptotic figures and mitosis [13]. Malignant forms tend to infiltrate local structures, such as muscle, tendon or adipose tissue [13].

Clinical presentation

As the typical patient with GCTTS is 30 to 50 years of age, this is a rare disease in the pediatric population, but its symptoms seem to be similar in both populations [13, 18]. The clinical presentation may vary, depending on tumor location. Tumor may appear as intra or extra-articular [7, 14]. When located in the hand, the GCTTS is usually an asymptomatic, painless, slow-growing and firm mass, frequently located on the palmar side [4, 16]. Sometimes it may cause swelling or periarticular effusion [4]. Pain and swelling are characteristic for the intra-articular location. When this tumor is located in hip joint, pain is the main symptom, with reduced swelling [14]. Among the significantly less frequent symptoms are: reduced range of motion in the joint, instability or locking of the joint, increased warmth of skin in the area of the affected joint. The extra-articular

presentation is usually associated with pain and noticeable tumor mass, while the swelling or joint malfunction appear significantly less often [14]. When located in the digits, the clinical picture may include mobility disfunction or pain [16]. Symptoms are usually present for 10 months to 3 years [14]. GCTTS may appear as a main tumor with satellite tumors located a few millimeters away from it [4, 16]. GCTTS of the knee may present similarly to meniscal syndrome [4, 14]. Tumor located in the spine is extremely rare, but may cause neck, shoulder or back pain, nerve root compression symptoms and limb disfunction, which are dependent on tumor size. [2].

According to Al-Qattan's classification, there are two types of GCTTS:

- **type 1** – single tumor surrounded by a round or lobulated capsule of various thickness;
- **type 2** – multiple separate tumors without clearly marked capsule [1, 16].

Type 2 is described as having higher recurrence rates than type 1 [1, 16].

Differential diagnosis should contain ganglion cysts, lipomas, inclusion cysts, fibromas, rheumatoid nodules, synovial sarcoma, pigmented villonodular synovitis or inflammation of tendon sheath, all of which may be easily diagnosed microscopically [13].

Diagnostic methods

Magnetic resonance imaging (MRI), regarded as diagnostic method of choice, allows to recognize precisely how the tumor is affecting its surroundings (e.g. nerves, joints or veins). It is also helpful when segregating tumors to types 1 and 2 in Al-Qattan classification. MRI can be also used as a tool to plan surgical treatment. Tumors appear as structures connected with tendon and its sheath, which is pathognomonic for GCTTS [1, 16, 19-21]. MRI scans of intra-articular tumors can also reveal joint effusion, synovial hypertrophy or pressure erosions [22]. Localized forms are well-defined, while diffuse forms usually appear as located in joints and associated with its effusion. Hemosiderin deposits present in some tumors are connected with weak to intermediate T1 and T2 signals, with enhancement after gadolinium injection [2, 19]. Nguyen et al. reported, that in MRI scans of 24 children, most of them presented joint effusion signs, and it was significantly more frequent in patients with diffuse type of tumor [22].

In X-ray images it is possible to notice the impression of a growing tumor on bone, particularly in the phalanges. Erosion of cortical bone, degenerative lesions, cystic erosions or soft-tissue masses may be also described [1, 2, 10, 16].

Doppler ultrasonography allows assessment of the tumor's vascularization. In most cases GCTTS is not vascularized

[16, 19-20]. Ultrasound-guided fine needle aspiration and cytology may also be performed as a part of the diagnostic process because in most cases its results correspond with histopathology findings [19, 21].

Treatment options

The gold standard of GCTTS treatment is total resection surgery with a careful preservation of tendons, arteries, veins and nerves in the local area of the lesion [1, 14]. In localized tumors a single surgical procedure is usually sufficient. If applicable, arthroscopy should be the method of choice, as it reduces morbidity and recurrence rate [14]. Diffuse GCTTS usually requires surgical excision. It may be problematic to obtain total resection of affected tissue, so recurrences may appear more frequently [14]. The most important issues regarding surgical management are joint damage, loss of function and recurrence [1, 7]. Risk factors of GCTTS recurrence are presented in Table 1 [1, 4, 16, 23].

Table 1. Risk factors of GCTTS recurrence

Most common risk factors of giant cell tumor of tendon sheath recurrence
<ul style="list-style-type: none"> • Incomplete excision • Joint or bone involvement • Type 2 (Al-Qattan classification) • Presence of mitotic figures • Male gender • Tumor size > 20 mm • History of GCTTS recurrence • Tumor's histological image

Cases of incomplete resection may need post-operative radiotherapy, to avoid recurrence lesions, according to Gouin et al. [19]. Radiotherapy may be applied as an external beam or intra-articular injection of radioactive isotopes [14].

The ENLIVEN study published in 2016 demonstrated that oral doses of pexidartinib (inhibitor of the colony stimulating factor 1 receptor, CSF1R) is effective in treating GCTTS. It is noteworthy that this study included only patients above 18 years of age and at this moment there are no published articles regarding its use in the pediatric population [12, 14, 23-24].

Pediatric cases of GCTTS

As noted earlier, GCTTS is indeed a rare disease in the pediatric population. In Table 2 we summarised selected case reports published in the literature [2, 13, 25-32].

In their study of 26 patients with GCTTS of foot and ankle, Cevik et al. included 6 children (see the summary in Table 3) [23].

Table 2. Selected pediatric cases of GCTTS summarized

Study	Number of cases	Sex	Location	Summary
Tsui et al. [27]	1	F	Spine	<ul style="list-style-type: none"> Spinal GCTTS of C1-C2 joint co-existing with medulloblastoma no resection due to lack of symptoms and technical difficulties
Mastboom et al. [6, 11]	76	39 F 37 M	Mostly big joints – knee, ankle, wrist	<ul style="list-style-type: none"> 29 cases of localized type 38 cases of diffuse type 9 unknown cases
Zeng et al. [2]	2	F	Spine	<ul style="list-style-type: none"> symptoms included weakness and numbness of limbs total resection was performed
Occhipitini et al. [13]	2	M, F	Toes	<ul style="list-style-type: none"> male patient had tetraploidy and chromosomal rearrangements female patient had normal karyotype
Ansari et al. [26]	3	F	Fingers	<ul style="list-style-type: none"> no bone erosion resection was performed
Meyers et al. [28]	1	M	Mandible and multiple joints	<ul style="list-style-type: none"> coexistence with Noonan syndrome Noonan syndrome is known to be connected with mandibular GCTTS, but it rarely occurs in other multiple joints
Shukla et al. [29]	1	M	Proximal phalanx of middle finger	<ul style="list-style-type: none"> no trauma history; painless swelling of proximal phalanx no bony erosion
Mankuzhy et al. [30]	1	F	Thigh	<ul style="list-style-type: none"> KRAS p.G12D mutation overexpression of CSF1R diffuse type of GCTTS
Yun et al. [31]	1	M	Shoulder	<ul style="list-style-type: none"> mass originating from the deltoid muscle intramuscular, diffuse type of GCTTS
Cho et al. [32]	1	F	Neck	<ul style="list-style-type: none"> tumor originating from the occipital condyle with erosion of the skull and atlas total resection of the mass with condylectomy, partial laminectomy of atlas and suboccipital craniectomy were performed

Table 3. Clinical details of pediatric patients with GCTTS located in foot and ankle according to Cevik et al.

Age	Sex	Size [cm]	Symptoms duration	Pre-operative diagnosis	Type	Recurrence after surgery
11	M	1 x 1 x 0.5	3 months	Ganglion	Localized	No
15	F	2 x 1.8 x 1.5	5 years	None	Localized	No
8	F	6 x 3 x 3	4 years	Hemangioma	Localized	No
8	F	4.5 x 3 x 2	2 years	GCTTS	Localized	Yes
17	M	4.4 x 3.5 x 2	2 years	None	Diffuse	No
10	F	3.5 x 1.5 x 0.7	3 years	Schwannoma	Localized	No

Their analysis revealed that most of the children presented symptoms for a few years before being diagnosed and surgically treated and pre-operative diagnoses were mostly wrong, only one patient was diagnosed correctly. Five out of 6 children with GCTTS had a localized type of tumor, and one had a diffuse type. Five of them did not present signs of recurrence after a follow up, and one female patient with a localized type of tumor manifested recurrence symptoms after 24 months, due to incomplete resection. There was no recurrence after re-operation. [23].

There are case reports describing GCTTS mimicking septic arthritis. This clinical situation is uncommon, but should be taken into consideration, as it may lead to unnecessary or inadequate surgical intervention [33-34]. According to the literature, such cases may be applicable in pediatric population, especially in patients with locomotory dysfunctions and elevated inflammatory markers.

Conclusions

Giant cell tumor of tendon sheath is relatively rare disease among adults, and even more rare in pediatric population. Pexidartinib appears promising in the treatment of GCTTS, however the ENLIVEN study did not include the assessment of its effect on children. It is worth remembering, that the knowledge about GCTTS is still limited and there is a need for diagnostic and therapeutic recommendations.

Funding

None.

Conflicts of interests

None.

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The application of 3D printing in neurosurgery: present and future

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Abstract

Background: For about 20 years we have been observing the development of three-dimensional (3D) printing. The aim of this study was to systematize the current knowledge on the use of 3D printing technology in neurosurgery and to attempt an outline the future paths of its development. **Material and methods:** The analysis was based on English-language literature from 2017-2021 indexed in the Mendeley and Scopus databases. **Results:** The application of 3D printing in neurosurgery concerns: 1) teaching students, 2) training of residents neurosurgeons, 3) individualized surgery planning, 4) dedicated cranial and spinal implants, 5) the future of 3D printing in neurosurgical implantology. There were 5 main neurosurgery subtopics in which 3D printing was used: "vascular neurosurgery" (31%), "skull, cranial neurosurgery" (22.4%), "neuro-oncology" (19.3%), "spine" (14.3%) and "others" (13%). The number of published articles has been steadily increasing by 11-33% annually. **Conclusion:** 3D printing has an enormous potential for clinical use and in the we will continue to observe its dynamic development. In neurosurgery 3D prints are currently most commonly used for didactic purposes as detailed anatomical models, for training residents and young surgeons and by specialists for the simulation of complex or innovative surgeries. The future of the use of additive 3D printing in neurosurgery lies in the biological 3D printing, the creation of artificial organs and the development of biological implants in tissue engineering.

Keywords: neurosurgery · 3D printing · bioprinting · anatomical models · operative training

Citation

Dzierżanowska N, Krakowiak M, Sokal P. The application of 3D printing in neurosurgery: present and future. Eur J Transl Clin Med. 2023;6(1):70-78.

DOI: [10.31373/ejtcmm/158565](https://doi.org/10.31373/ejtcmm/158565)

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Available online: www.ejtcmm.gumed.edu.pl

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Introduction

In recent decades we have observed a dynamic, even aggressive, introduction of modern technologies into medical practice. This includes computerization, implantology, transplantation, new techniques of macroscopic and microscopic imaging, endoscopy, navigation and finally the introduction of surgical robots such as Da Vinci systems, ROSA (Robotic Surgical Assistant) and others [1-3]. Altogether technology has opened new possibilities for modern medicine, science, teaching, standardization and repeatability of surgical techniques, shortening the operation time, increasing its safety and many others. The exact same benefits can be derived from the introduction of three-dimensional (3D) printing in neurosurgery. Simple anatomical models of individual organs made of clay or plaster have been known for hundreds of years and were used mainly as teaching aids. The 1980s marked the beginning of the development of 3D printing (at the time it was called stereolithography) pioneered by Charles Hull [4] mainly in the automotive and aerospace industries. The end of the 1990s witnessed the first 3D models of intracranial vascular pathologies (aneurysms and arteriovenous malformations) created for educational purposes [5]. Further development of computing power, modern methods of 2D radiological imaging, software-generated 3D images, printing of such models and the development of new materials imitating physical properties of human tissue has opened almost unlimited possibilities of using 3D models in science, education, surgical training or intraoperative implantology [6-14].

The aim of this study was to analyze both quantitatively and subject-wise the scientific literature concerning the use of additive (3D) printing technology in neurosurgery over the past 5 years, to demonstrate the trends in the use of this technology and its potential paths of future development.

Material and methods

We have analyzed the English-language full-text papers available in Scopus and Mendeley databases. One independent Author (ND) searched the databases using the keywords “neurosurgery” and “3D” and “print” or “printing” used in the title and/or abstract [15]. We narrowed the search to articles published in 2017-2021 with the following inclusion criteria: complete English-language text, original article, review paper, case report. Publications written in a language other than English, lacking full text, repeated in different scientific literature

databases, not relevant to the topic, conference reports and book chapters were excluded from further analysis. We then performed a quantitative and qualitative analysis of the eligible papers. In the quantitative assessment, we calculated how the number and topics of the articles have changed over the years. For this purpose, consecutive keywords “tumor”, “vascular”, “aneurysm”, “malformation”, “spine”, “skull” and “cranial” were entered. In the qualitative analysis we assessed didactic and practical use of 3D printing by medical students, neurosurgery residents, specialists and researchers as well as weaknesses and most importantly, the future of 3D technology in neurosurgery.

Results

Quantitative evaluation

We initially found a total of 191 articles in the Scopus and 153 in the Mendeley database. Restricting the year of publish to 2017-2021, these values were respectively 141 and 94, resulting in a total of 235 papers on the use of additive printing in neurosurgery. Following the PRISMA protocol [15], applying the inclusion and exclusion criteria described above, a total of 140 articles were included for further detailed analysis. The number of published articles has been steadily increasing by 11% to 33% per year (Fig. 1).

Articles were mainly written by authors from countries with a high index of technological, IT and industrial development: the United States, China and Germany. Despite the explicit keywords applied, the search yielded articles covering quite a few scientific disciplines including medicine, computer science, materials science, biochemistry, engineering, genetic engineering, etc. This made the study topic interesting and challenging because it required selection of the articles to those with medical application.

Subject evaluation

There were 4 main neurosurgery subtopics in which 3D printing was used: neuro-oncology, cerebrovascular anatomy and pathology (aneurysms, arteriovenous malformations),

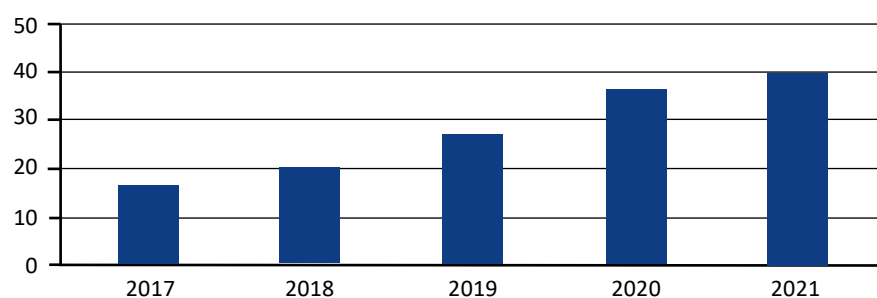


Figure 1. The number of articles about “neurosurgery and 3D printing” successively increased in the years 2017-2021

spine implantology and reconstruction of the skull bone defect. Some of the articles covered several topics in the use of 3D printing, hence quantitative percentage relationships were presented rather than absolute numbers. There was also an additional group “others” which accounted for as much as 13% of all topics and was dominated by such subjects as biopsy, insertion of DBS (deep brain stimulation), endoscopy, anatomy of nervous structures, morphometry, and tractography. Out of 140 articles we analyzed, 31% were focused on “vascular neurosurgery,” 22.4% on “skull, cranial neurosurgery,” 19.3% on “neuro-oncology,” 14.3% on “spine” and 13% concerned “others” (Fig. 2 and 3).

In the data we analyzed, it was possible to identify 6 themes that dominate the “neurosurgery vs. 3D printing” research. These are: 1) teaching of medical students, 2) training of residents and young neurosurgeons, 3) surgical planning, 4) custom/individual implants, 5) disadvantages of 3D printing and areas for improvement and 6) future developments in 3D printing development (near and distant future).

Discussion

In the literature we analyzed there was a significant increase in the number of articles on the topic of “neurosurgery vs. 3D printing” with 17 articles in 2017 and 40 in 2021 (11-33% increase per year). This confirms the general opinion about the interest of the science and industry communities in this topic, the progressive development of additive printing technology and its clinical or didactic usefulness. The largest amount of studies was related to “neuro-oncology,” “cerebrovascular” and “skull” and these are mainly models for training, surgery planning, new applications etc. [8, 10, 16-22]. This was a bit of a surprise, as other surgical specialties (e.g. general surgery, maxillofacial surgery) or

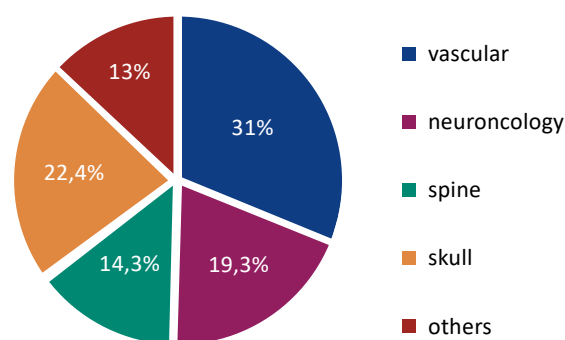


Figure 3. The percentage of “3D printing in neurosurgery” work in the context of the 5 main subtopics for the analysed time period 2017-2021

dentistry are dominated by the topic of implantology and intraoperative use of models [23]. It would seem that it should be analogous in neurosurgery (spinal implants, dedicated interbody cages, stabilizing screws) [24-30]. The reason for this discrepancy may lie in the potential costs of such implants, which are so far higher than the standard spinal surgery implants [24-25, 30-32]. The same applies to the cost of printing cranial defects in reparative surgery compared to intraoperative manual fabrication of such defects from a plastic mass (polymethyl methacrylate, PMMA) [11, 14, 19, 33]. Whereas the guides and components of dental implants are made of fairly simple materials that are not subjected to the same forces as spinal implants. Moreover, they are printed quite commonly, so their cost is lower compared to the cost of neurosurgical implants. In general, the issue of cost is one of the disadvantages of 3D printing and is discussed later in the paper. We did not separate the articles about pediatric neurosurgery, as there were only 15 articles regarding this

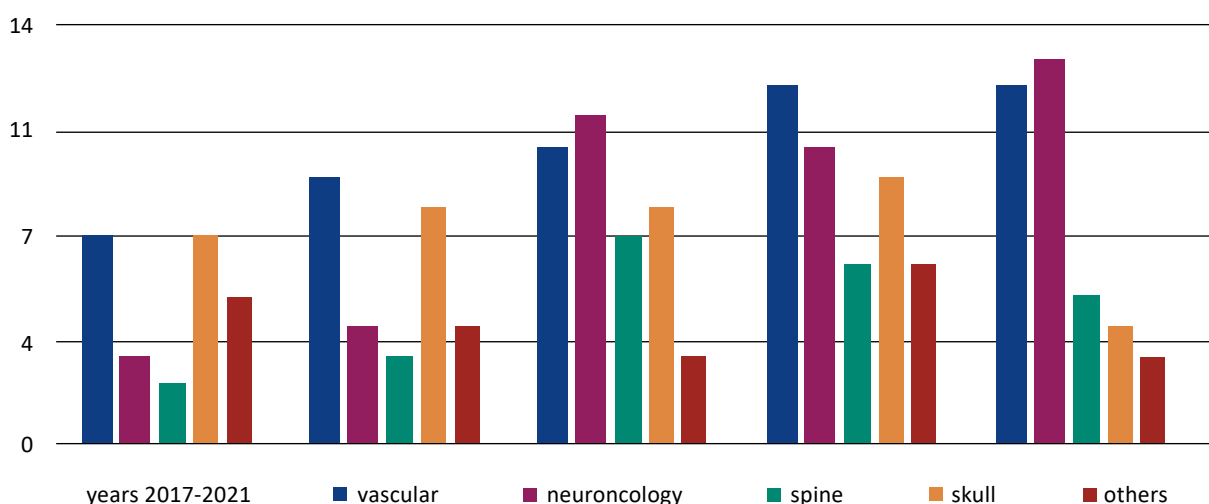


Figure 2. The number of articles focused on the 5 main subtopics published in each year from 2017 to 2021

patient group and were focused on training for brain tumor biopsy, ventriculocisternostomy in hydrocephalus, treatment of skull deformities in the course of craniosynostosis [7, 11-12, 14, 20].

Medical student education

The educational usefulness of 3D prints in medicine has been observed ever since their introduction [6, 34]. As their shapes and availability improve, the market is actively growing. This is due to the cost of such models, which are reusable and can last for years, the limited number of professional anatomy laboratories, the limited number of human models to teach medical students. It is important to bear in mind the ever-increasing number of students, not only of medicine and dentistry, but also of other health sciences e.g. physiotherapy, radiology technology, dietetics, nursing sub-specialties. Let's not forget the fine arts and physical education students.

Training of residents and young neurosurgeons

For quite many years it has been possible to notice a change in the type of training of residents from the form of gaining experience in the operating room to maximizing learning on phantoms and 3D radiological images. This is obviously due to ethical (ensuring patient safety) and legal reasons. The use of reconstructed images of MRI, CT examinations for this purpose is very useful, but not sufficient. It is the printed spatial models that help teach how the position of anatomical structures will change when the angle of view of the surgical field shifts during aneurysm clipping or how to safely bypass eloquent fields (motor and sensory pathways) in brain tumor resection [8, 16-17, 20, 35]. They can also be used to practice simple surgical procedures like biopsy, craniotomy or spinal canal decompression [19, 29-30, 37]. Moreover, 3D Prints help clarify the communication with patients, e.g. explain the surgical procedure and obtain the informed consent. [10-13, 28, 33-36].

Planning operations, new techniques and surgical accesses

Regular training of the acquired skills is necessary to maintain high professional qualifications of the neurosurgeon. For this purpose, during courses neurosurgeons can perform simulated operations either on fresh frozen human cadavers or anaesthetized animals (e.g. pigs). 3D phantoms can significantly help to organize such courses. In addition, the surgeon can plan the individual steps of the operation based on a specific case after preparing an additive model of a tumor, aneurysm or the patient's spine [8, 11-13, 18, 25-27, 38-39]. Here we enter the domain of personalized medicine, dedicated to a specific patient. Such models can also serve

as material for research on modifying the surgical approach or even developing innovative spinal or cranial repair procedures [16, 21-22, 31, 40].

Intraoperative use of 3D implants

Currently in the literature, the use of additive printing in neurosurgery focuses on cranial bone repair surgeries and much less frequently on spinal surgeries [14, 25-26, 41]. Restorative operations include craniosynostosis-related procedures in the pediatric patients and in adults, the replenishment of the cranial bone after a craniectomy [11, 14, 41]. Currently, in the intraoperative setting the neurosurgeon prepares a specific fragment by hand from PMMA mass and attaches it in place of the defect [41-42]. In children, various proprietary material such as ribs are used. Intraoperative preparation of the synthetic component requires time, which may increase the overall cost of surgery and the final cosmetic results are not as good as with a computer-prepared implant. Based on a preoperative CT scan of the skull bone (mirror-image), 3D files of the missing bone fragment are prepared, which can then be printed and transplanted to the patient after sterilization [11, 14, 31, 33, 41-42] – see the Fig. 4. The downside of course is still the cost, as mentioned below [41, 43].

The near future of 3D printing

Short-term developments are consequences of today's limitations of the 3D printing technology: cost, print quality, preparation time and material available. The cost of printing ranges from a few tens of US dollars (USD) to several thousand (these are the costs of materials, software, processing, human labor by computer scientists, engineers, technicians, doctors, etc.) [43]. Currently, this is one of the main factors limiting the widespread clinical use of this technology. The cost of the 3D printer itself is an expense of several to several hundred thousand USD, not to mention the costs of hardware, software, printing material and involvement of people in the production of the final product. The authors emphasize that the widespread use and availability of 3D printing technology will drive the further development of the hardware and software as well as the decrease in costs on the basis of competition. It must be remembered that models have a limited life span due to wear and tear during training and must be successively replaced [19, 44].

Further 3D print development depends on materials used and thus requires improvement

The development of, bioengineering, creation of new materials imitating the physical properties of human tissue and organ fragments, printing with multiple materials simultaneously, must go hand in hand [45-46]. Further development of increased resolution of computed tomography (CT), magnetic

resonance (MR) and other examinations is necessary [46-47]. The time of print preparation ranges from several hours to even a few days. It is crucial to reduce this time in order to increase the neurosurgeons' interest in using 3D prints for scheduled and in urgent operations, e.g. intraoperative printing in case of post-traumatic skull bone defects. This creates the need for technical development of printers, fast computer processors as well as durable, efficient mechanical components [47]. Another element is the involvement of IT specialists, radiologists, technicians, neurosurgeons (e.g. residents) within the hospital to introduce procedures that will allow rapid implementation of treatment offered by 3D printing. The scope of printing is most often limited to a given organ or anatomical structure and is devoid of adjacent tissues and structures that are of colossal importance during surgery. Thus, further development should aim towards printing entire models with simultaneous use of multiple materials, something along the lines of a print that is done on an already printed part (print-in-print-out) [45].

The distant future of 3D printing

The 3D printing has great potential to revolutionize personalized medicine, i.e. individualized treatment for a specific patient, especially in the field of implantology. In the case of neuro-traumatology, this is already happening (cranial bone defects, spinal implants for stabilization, artificial discs). However, looking into the future, the most promising route is that of bioprinting of complex tissues, molecular printing, so-called "living" implants. That is, on the one hand, targeted stimulation of stem cell growth into a specific cell line and, on the other hand, biomatrices on which the growth and development of tissues or organ fragments will be postulated [48-50]. Currently, it remains at the laboratory stage and in order to have a clinical application many elements need to be improved (or developed), e.g. increasing the resolution to the sub-cellular level (moving on the nanometric scale) [49-51]. Another challenge is the viability of bioprints, a suitable interface with sufficient mechanical properties

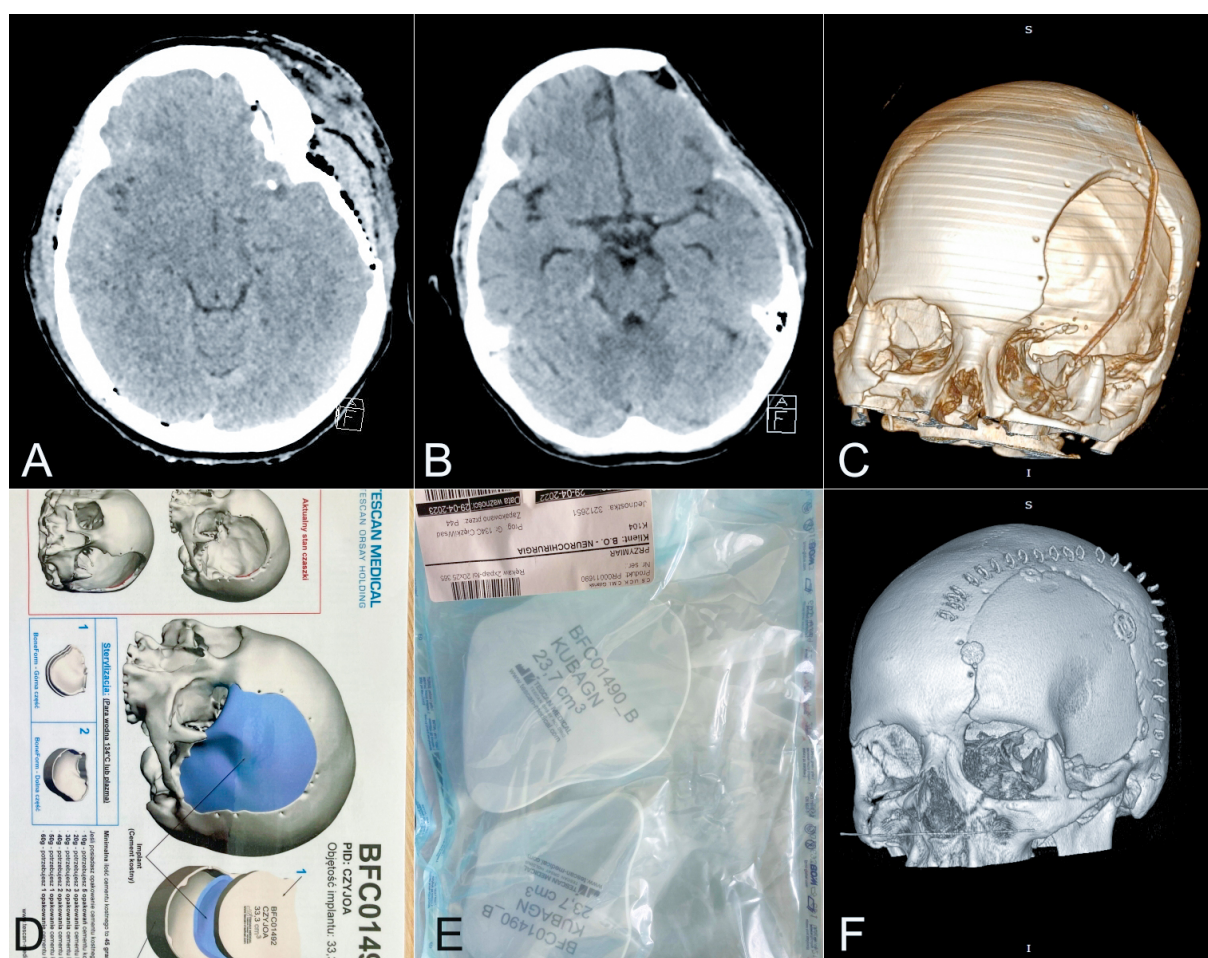


Figure 4. A) CT scan of the head (transverse section) – patient age 29, craniofacial trauma, fracture of left temporal and frontal bone with compression of the brain tissue and subdural hematoma, B) follow-up head CT after decompression surgery (in this case a fronto-temporal craniectomy), C) 3D reconstruction of the skull with a visible post-craniectomy bone defect, D) package with the material for 3D printing of cranial bone flap, E) 3D prints of cranial bone cavity flap prepared for sterilization, F) 3D reconstruction of the skull after surgery cranioplasty

to allow adequate interaction with the rest of the tissue or organ. This interaction concerns activities such as blood flow, growth, oxidation, neurological stimulation, secretory, functionality, etc. [50, 52]. Another expected advance is 3D in situ printing, which involves real-time fabrication of specific tissues during surgical intervention. Here, developments in engineering, computer science are needed to achieve printing speeds for utility [49, 51].

Limitations

One of the limitations of our analysis is that the articles generally emphasized the clinical, intraoperative utility of 3D printing, which we also cite. It would be worthwhile to expand the research on this issue with a meta-analysis of clinical papers to find out whether and by how much the time of surgery decreased, by how much the cost of surgery actually changed, to what extent the use of 3D prints increased the safety of surgery or reduced the complications. Most papers are dominated only by the surgeon's subjective feelings. Such an analysis is difficult because clinical papers cover very heterogeneous cases (central nervous system tumors, spine instrumentation procedures, peripheral nerve treatment) and low repeatability of cases. Another issue concerns the future of bioprinting, i.e. the use of tissue that imitates the properties of living tissue. However, this topic has been described laconically in the reviewed papers as the future rather than an advancement of current research. The programming of tissue culture matrices or other bio-elements, the use of induced stem cells are mentioned [48]. The problem itself is extremely perplexing and worth extending. However, the literature selection protocol we used (PRISMA) limited our analysis only to already existing applications in neurosurgery or "smuggled out" some futurological issues [15]. Thus, it is worthwhile to focus separately only on the topic of "future of 3D printing applications" and analyze articles in the field of tissue engineering, genetic engineering, molecular biology,

transplantology, etc. Moreover, Further work of highly-qualified specialists in complex multidisciplinary teams is crucial to ensure the development of the 3D printing technology, because the knowledge of medicine seems to be clearly insufficient for such a complex task.

Conclusions

Based on our analysis of the literature, five basic conclusions can be drawn regarding 3D printing in neurosurgery:

1. It has an enormous potential for clinical use and the constantly increasing number of published studies about it confirms that in the upcoming years we will see a dynamic development in this field.
2. Research on 3D printing in neurosurgery is most often focused on neuro-oncology and cerebrovascular diseases.
3. Currently in neurosurgery, 3D prints are most commonly used for educational purposes as detailed anatomical models, for training residents and young surgeons and by specialists for the simulation of complex or innovative operations.
4. The future of the use of additive printing in neurosurgery lies in implantology and the spine and skull reconstruction fields are the most advanced in this matter.
5. The development of biological 3D printing, the creation of artificial organs, the advancement of biological implants (tissue engineering) may bring the greatest clinical benefits.

Funding

None.

Conflicts of interests

None.

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The Ukrainian refugee crisis: its ethical aspects and the challenges for the Polish healthcare system – a descriptive review

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Abstract

Since the start of the Russian Invasion of Ukraine on 24.02.2022, millions of people (mostly women and children) have fled Ukraine. Majority of them fled to Poland and providing adequate medical care for the massive influx of refugees is a considerable challenge for the Polish healthcare system, already burdened by the COVID-19 pandemic and staff shortages. The purpose of this study was to identify: the potential health problems of the incoming population, the legal and ethical aspects of the refugee crisis and its implications on public health. Combining the current data with previous research on refugee crises reveals a set of issues that need to be addressed. Ensuring continuity of chronic disease treatment, mental health, the risk of spreading vaccineable preventable diseases, high rates of tuberculosis, HIV and the ongoing COVID-19 pandemic are among the main concerns. In the near future, answers will have to be found to the emerging ethical questions of equal, safe access to care, implications of the language barrier, immunisation coverage, medical staff shortages, and existing legal and ethical regulations. As the emergency responses are addressed, the hosting countries need to prepare long-term resolutions.

Keywords: ethics • public health • Ukraine • refugee crisis • Polish healthcare

Citation

Kolińska K, Paprocka-Lipińska A, Koliński M. The Ukrainian refugee crisis: its ethical aspects and challenges for the Polish healthcare system – a descriptive review. Eur J Transl Clin Med. 2023;6(1):79-86.

DOI: [10.31373/ejtcmm/162233](https://doi.org/10.31373/ejtcmm/162233)

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Available online: www.ejtcmm.gumed.edu.pl

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Introduction

Despite the fact that the war in Ukraine has been going on since February 24th 2022, to date only a few articles have been published regarding the health problems of Ukrainian refugees [1-2]. The United Nations High Commissioner for Refugees (UNHCR), World Health Organisation (WHO) and particular non-government organisations have regularly published reports providing important information elucidating the scale of this crisis and analysing the problems arising in connection with it [3-5]. Furthermore, the data allows us to look for solutions derived from knowledge gained during previous refugee crises [6]. For countries such as Poland, inexperienced at managing large-scale intake of refugees, this is a new situation to cope with, not disregarding its inevitable ethical and legal aspects.

The 1951 Geneva Convention states that all refugees should be guaranteed the same rights as the citizens of the host country. If this is not possible, this vulnerable group should be privileged in comparison with other categories of foreigners residing in the particular country [7]. Guaranteeing an adequate level of access to healthcare is a considerable challenge for host countries, already burdened by the COVID-19 pandemic and staff shortages [2]. While there is a need for emergency measures in case of immediate health threats, there also appears a need for continuity of treatment and long-term solutions. To find such most effective solutions it is crucial to accurately identify the scope of this crisis, the issues that accompany it and manage them effectively.

Material and methods

To find the data necessary for this review, we collected scientific publications and retrieved statistical data and information from governmental institutions as well as non-governmental international organisations. Our search was conducted through publications and sources available in English and Polish language. We focused on including in the analysis publications regarding challenges for refugees and the subsequent challenges to the healthcare system. Next, we confronted the above mentioned findings with the statistical data regarding the current Ukrainian refugee situation. We also reviewed the reference lists of selected publications for additional sources, applicable ethical and legal regulations and further internet resources.

Results

Finally, we included in our analysis 12 full text articles and 19 reports from international organisations.

Discussion

Scale of the problem

The influx of refugees from Ukraine to Poland has been consistent since the start of the Russian invasion. Although specifying the exact number of refugees from Ukraine in Poland continues to pose a challenge, one way of estimating the scale of this matter is by looking at the governmental data: over 1.5 million applications for foreigner status have been registered so far [8]. Of these 1.4 million, 69.5% of applicants are women and an additional 19.4% are males under 18 years old [8]. This corresponds to the data presented by WHO stating that approximately 96% are women and children [9]. Moreover, the data recorded by the UNHCR regarding the applications of refugees from Ukraine registered for temporary protection programmes (1.4 million) [10] is in accordance with the data presented by the Polish national registry.

However, we must bear in mind that a significant number of Ukrainians will not be included in the above-mentioned records as they may perceive Poland as a place of temporary asylum before returning to Ukraine once they consider it safe or utterly necessary. For instance, according to data from the WHO, as of August 11th 2022 around 16.6 million refugees have been documented crossing the border between the two countries [11]. It is substantial to understand that this figure can be falsely magnified as it includes multiple crossings by the same person, yet it does not include refugees that have arrived in Poland indirectly through its neighbouring countries such as Slovakia or Czech Republic, nor does it take into account illegal crossings. Nevertheless, it is clear that not all of the refugees have remained in Poland. Once having crossed the Schengen area it is difficult to track them. Overall, Poland has been the main destination among the refugee-receiving countries in the first months of the crisis [3] as also illustrated in figure 1.

Identification of major health problems

It must be noted that refugees brought their health problems with them, therefore apart from emergency relief, it is expected that their illnesses will mainly burden the health system [6]. The WHO identifies the main health problems that are very likely to increase mortality and morbidity [12]. The “immediate health risks” noted among the refugees from Ukraine are COVID-19, measles, chronic infectious diseases (tuberculosis/HIV/HBV/HCV), cardiovascular disease, chronic respiratory diseases, diabetes as well as mental health problems such as depression, anxiety and post-traumatic stress disorder (PTSD) [12].

Non-communicable diseases (NCDs) are a primary cause of mortality (91% of total deaths) in Ukraine, led by cardiovascular diseases (66%) and followed by cancer (13%), diabetes,

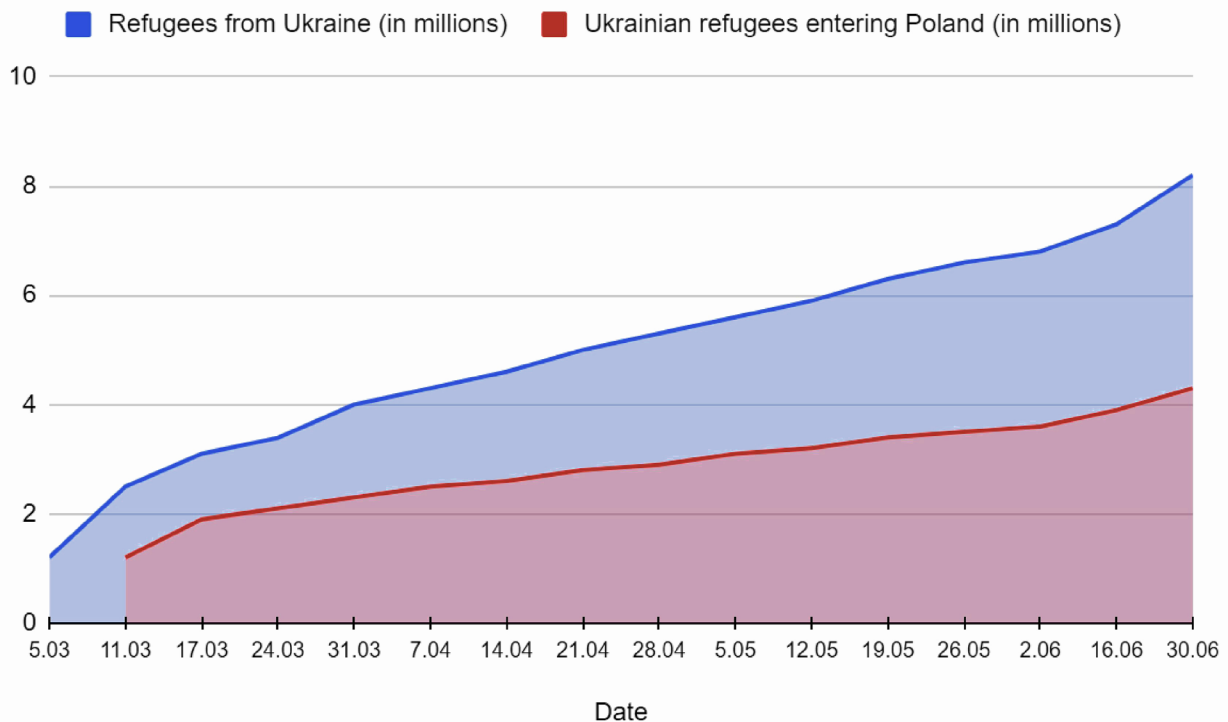


Figure 1. Total number of refugees from Ukraine and Ukrainian refugees entering Poland from 5.03 to 30.06.2022. Data from 16 WHO reports (5.03-30.06.2022): “Emergency in Ukraine: external situation report.” Available at: <https://www.who.int/europe/emergencies/situations/ukraine-emergency/situation-reports-global>

chronic respiratory disease and mental health conditions [13]. In a survey conducted by the International Organisation for Migration, around 1 of every 5 respondents indicated that they or someone within their family needed to discontinue their medication by the start of the war; 85% of which stopped due to lack of availability of medication and 44% because they could not afford it [14]. Interruptions in treatment due to lack of availability or during transit can be fatal.

Mental health disorders are another significant problem to which refugees are particularly exposed. There is a low mental health awareness and a considerable level of stigmatisation in the Ukrainian population [12]. Before the war, only 3.2% of people suffering from depression received treatment [12]. 12.4% of Ukrainians had symptoms equivalent to a diagnosis of clinical depression, whereas suicide accounts for 2% of mortality in Ukraine [12-13]. Another concern is the rate of alcohol use disorders, four times higher than the global average (respectively 6%; 1.5% population prevalence), which can be intensified by trauma [13]. The risk of developing PTSD among refugees is very high [6]. Before the full-scale conflict, the risk of PTSD among people internally displaced in Ukraine was 30% [15]. In war-experienced populations, it is estimated that 1 in 5 people (22%) will have depression, anxiety, PTSD, bipolar disorder or schizo-

phrenia [16]. An especially vulnerable group consists of the double displaced group (people displaced after having been already once displaced internally in Ukraine) [15]. Regrettably this data does not allow us to discern further between particular populations such as soldiers. In the upcoming months, an even greater increase of challenges regarding mental health is predicted: a surge of acute psychological distress cases, addiction problems, aggravation of chronic mental health issues along with lack of psychosocial support.

Furthermore, vaccine-preventable and chronic infectious diseases are rising concerns. Ukraine faced an epidemic of measles from 2017 to 2020. Even though measles vaccination coverage rose from 45% in 2016 to 87% in 2021, it is still below the recommended 95% population threshold [13, 17]. Ukraine has also not achieved this level of vaccination coverage for any of the core vaccinations: BCG, DTP3, Pol3, MCV and HepB3 [18]. Of particular concern is the polio outbreak of circulating vaccine-derived poliovirus type 2 (cVDPV2) with 20 cases identified in two regions of Ukraine in 2021 [19]. Thus, since vaccination coverage in Ukraine is among the lowest in the region, Poland and other host countries should take steps to prevent the spread of diseases preventable by vaccination of both their own and refugee populations. This is also of particular importance as 38.6% of Ukrainian refugees

registered in Poland are below 18 years old [8].

In the WHO European Region refugees are at a higher risk of tuberculosis (TB) and there are inequalities in multi-drug-resistant tuberculosis (MDR-TB) ratio compared to the host populations [6]. Experience from other armed conflicts has shown that there is a risk of increasing TB cases and mortality [20]. This can be attributed to easier transmission in crowded settings, malnutrition interruptions, lack of or inadequate treatment, reactivation of latent infections and limited access to health care [20]. Ukraine presents a distinctively high TB rate worldwide, with an estimate of almost 30,000 new cases every year [21]. Globally, it is among the top 10 countries with the most elevated number of MDR-TB and it ranks as the second highest regarding the prevalence of HIV-TB coinfection (26%), when considering the WHO European Region [21].

HIV is another public health issue in Ukraine, with the second-highest prevalence within the WHO's European Region [22]. There is also a disparity in treatment coverage, 57% in Ukraine compared to 82% in the EU, according to the WHO [21-22]. Refugees and migrants more often are diagnosed at a later stage of the HIV infection [6]. 44% of new HIV diagnoses in the WHO European Region in 2020 were reported in individuals who originated outside of the reporting country [22]. However, there are indications that a significant proportion of refugees and migrants, including those from countries with high HIV prevalence, become infected after their arrival in the WHO's European Region [6].

The overlapping of the COVID-19 pandemic with the war makes the situation even more difficult. The Ukrainian population has a substantially lower uptake (35%) of a primary COVID-19 vaccination series in the total than the 72.7% EU/EEA (European Economic Area) and Polish 59.6% average [23-24]. As the war started, around 5 million COVID-19 cases have been identified with a fivefold increase in detected SARS-CoV-2 infections between January and February 2022 [21, 25]. While there have been several waves of coronavirus infection incidence [21, 25], it must be considered these numbers immediately preceding the start of extensive migration towards neighbouring countries in Europe in addition to low percentages of vaccine uptake can result in a more severe course of the disease in unvaccinated individuals and a more dynamic transmission of the virus, further burdening the healthcare system. However, in the current situation, with eased restrictions in the neighbouring countries, it is difficult to predict what the real numbers are.

Ethical and legal aspects

The first regulations concerning the refugee issue appeared in the world after the Second World War. In 1951 the Geneva Convention (Refugee Convention) was adopted, which in 196 was supplemented by the New York Protocol, which in turn extended the Convention to non-European

countries [7]. Poland adopted both documents 40 years later, in 1991 [26-27]. Since the adoption of the convention, many economically developed countries have been targets for populations leaving their countries of origin. Accordingly, there is a "non refoulement" principle, described in Article 33, which states that "No Contracting State shall expel or return (*refouler*) a refugee in any manner whatsoever to the frontiers of territories where his life or freedom would be threatened on account of his race, religion, nationality, membership of a particular social group or political opinion" [26]. This consequently means taking measures to provide refugees with adequate protection and assistance by the state of their choice. The UNHCR reports have highlighted specific social and health problems of refugees, but also the impact of an increased population from other areas on the health security status of the population in the hosting country. It seems that given the particular threat of infectious diseases, it is reasonable to develop and use screening protocols for adults and children, additionally implementing vaccination programs to prevent epidemics. However, screening programs as well as the use of the resulting data may be controversial from an ethical point of view in the context of refugee and immigrant rights [28].

According to the Act on Patients' Rights and Patient Rights Ombudsman of 6th November 2008 – every person who reports to an institution providing medical services in Poland should receive medical assistance, in the light of the definition of "patient" in the Act, to which there are no additional criteria [29]. There is a lack of regulations concerning the process of provision of health services in the case of people who speak, for example, a language other than Polish. The main difficulties include collecting medical history and exercising the patient's right to information, as the information should be provided to the patient in an understandable way. There are no regulations stating explicitly whether the presence of an interpreter is indispensable.

In many cases, inviting an additional person who speaks the Ukrainian language into the interaction between the patient and the medical team has proved to be a practical and immediate solution. Furthermore, if the interpreter is a healthcare professional, then patient confidentiality is preserved. However, if this is not the case, the issue of protecting the patient's confidentiality needs to be considered. Another solution could be the use of ready-made documents – for example, the medical history sheet translated into Ukrainian, available on the website of the Polish Chamber of Physicians and Dentists [30]. These materials were posted in early March 2022, intended primarily for doctors and dentists working in hospital emergency departments and in medical institutions in border regions. In addition, the Ministry of Health has launched an application, which helps patients to communicate with medical staff in Polish, Ukrainian, Russian or English [31]. Some further tools are publicly available multilingual applications such as Symptom-

ate (after describing their symptoms users are given instructions on where they should go to get appropriate help) [32] or search engines maintained by medical organisations, e.g. the Polish Society of Family Medicine [33].

The need to provide services to a patient who does not understand Polish carries a risk of committing a medical error arising from the lack of clear communication. In addition to discussing the treatment plan formally, the patient's informed consent to provide health services is needed. A number of questions arise in relation to this issue: should the informed consent for medical service for a refugee be translated by a sworn translator? Can an informed consent form be accepted in a foreign language? Can the consent be signed on a form prepared in a language not understood by the patient? In the legal act regulating the practice of the medical and dental professions, there are no additional references concerning the above-mentioned issues [34].

The Polish Code of Medical Ethics, the document regulating the ethical side of practising the medical profession, contains general references to acting in the best interests of the patient, regardless of age, sex, race, genetic endowment, nationality, religion, social affiliation, material situation, political views or other conditions [35]. There are no descriptions of additional duties of the doctor and dentist regarding people arriving from foreign countries due to danger. In the United States of America, regular discussions have taken place within the structures of The American Medical Association which published resources for practising physicians regarding interpreting the provisions of the Code of Medical Ethics in relation to the medical care for patients who are immigrants, refugees or asylum seekers [36]. Such discussion certainly is necessary in the structures of the Polish health care system, taking into account the protracted state of war in Ukraine.

The question of guaranteeing equal access to health services in a situation of organisational difficulties and deficits in public health care remains to be thoroughly worked out. The legislature adopted on March 12th 2022, a law on assistance to citizens of Ukraine in connection with the armed conflict on the territory of their country and importantly, this legislation entered into force retroactively from February 24th 2022 [37]. There were additional provisions in the existing legislation – an amendment to the Act on Doctor and Dentist in art. 77, art. 85 (by adding provision that doctor or dentist provides health services to persons whose stay in Poland is legal) concerning reimbursement of medicines and art. 86 regulating the work of nurses and midwives. In practice, however, there arise specific problems as, for instance, a situation when a Ukrainian citizen requires medical assistance yet the medical personnel cannot recognize their entitlement to a reimbursed health service.

At present, the introduced solutions seem to have worked properly through mechanisms such as disinterested willingness to help, common sense in providing health

services despite difficulties in communicating with patients and the goodwill of both parties. In order to prevent possible abuses, the situation will certainly require long-term solutions which above all must address the key ethical question: how to reconcile the well-being and safety of the host community with the wellbeing and health security of an increased wave of refugees?

An attempt to identify long-term problems

The war in Ukraine has been going on for more than one year, whereas the experience with previous armed conflicts suggests no prompt resolution. The role of international organisations is crucial to contribute coordinated help. Non-governmental organisations such as Médecins Sans Frontières and the International Red Cross are supporting the management and logistics of reception centres and providing medical and shelter-related supplies in Poland as well as other countries neighbouring Ukraine [38-39]. The WHO together with other international agencies developed initiatives aimed at coordinating their efforts, such as the Refugee Health Extension, strengthening access to health-care [40-41]. Similarly, the European Center for Disease Control (ECDC) has developed guidelines for disease surveillance [42] while WHO delivered large quantities of medical supplies, e.g. antiretroviral drugs to Poland or PCR reagents to Romania [40].

Overall, an equally relevant task pertains to the refugee-hosting countries as they need to prepare for long-term support and the challenges that come with it.

A question arises whether we are able to guarantee a responsive and safe access to medical care without discrimination for both Ukrainians and refugees originating elsewhere? The available literature indicates that refugees and migrants, despite often initially being in better health than host populations, became more susceptible to illness over time [6]. Such a circumstance is attributed to inequalities and barriers to accessing healthcare, social determinants of health including poor living and sanitation conditions, low vaccination coverage, exposure to communicable diseases and disruptions in continuity of care [6]. It is therefore important to guarantee refugees prompt and accessible healthcare in the host countries.

Large migrant populations moving in crowded conditions can easily spread communicable diseases. During other crises, such outbreaks have been observed in migrant populations, including outbreaks of vaccine-preventable diseases in migrants from Eastern Europe [43]. In addition, given the low levels of immunisation and the high vaccination hesitancy in Ukraine along with the COVID-19 pandemic, widely available immunisation programmes must be guaranteed to ensure the safety of refugee populations and host countries. Then, another question arises whether it is ethical for us to require mandatory vaccinations for refugees?

Guaranteeing an adequate level of extemporary care along with continuing long-term therapy is only possible with an adequate proportion of medical staff. With almost half of the refugees in Poland being children [8], there has been a significant increase in the paediatric population that needs care, thus it seems that paediatricians and general practitioners will be particularly burdened. Poland has among the fewest of physicians per population in the European Union (EU), just 2.4 practising doctors per 1000 population and it is the second-lowest in Europe behind Turkey [44]. Lewtak et al. estimated that 2300 physicians and 5000 nurses per every million of incoming refugees are needed to maintain current the statistics [2]. Nonetheless, how to manage sufficient care with the existing shortage of medical professionals? Facilitating a pathway for the recognition of qualifications appears as a solution, but questions emerge as to how qualifications, in particular language competences, should be recognised. Such a solution, to be ethically justified, should be the same for all refugees regardless of their country of origin.

Furthermore, according to the existing literature the main issue identified by both refugees and healthcare providers is the above-mentioned language barrier [45]. This poses a considerable obstacle at every stage of medical services provision. Hence, the need for interpreters and translators is acknowledged.

Among other challenges mentioned by health care providers are time limitations, lack of competence and skills, emotional burden and cultural differences [46].

The geographical and cultural proximity to Ukraine seems to reduce the latter yet, it remains significant [47]. Poland and other host countries shall find the optimal solutions on how to respond to the health needs of refugees and the following relevant questions.

Conclusions

Providing medical care for refugees from Ukraine poses a completely new challenge for the Polish health care system. The outbreak of the war in Ukraine on February 24th 2022 and a rapidly growing wave of refugees crossing the Polish-Ukrainian border revealed deficiencies related to providing health services for the incoming population, which were and still are mainly women and children. While there was a need for emergency measures in case of immediate health threats, there also appeared a need for continuity of treatment and long-term solutions.

Funding

None.

Conflicts of interests

None.

Editor-in-Chief's Commentary

Ukraine is very important to us. Just as Poland had in the past, Ukraine is currently defending itself against an attack and fighting for its freedom, independent identity and full independence. The large number of Ukrainian citizens (mainly women and children) who found refuge in Poland, presented an unprecedented challenge for the local health care system. Providing both inpatient and outpatient care to refugees from Ukraine in the face of the crisis, we gained experience and learned lessons that ought to be shared with others.

For this reason, we decided to publish the article "The Ukrainian refugee crisis: its ethical aspects and challenges for the Polish healthcare system – a descriptive review." Although it is outside the scope of our Journal, this article contains an analysis of a timely and important problem of the modern world. According to the authors, this particular crisis revealed major deficiencies related to the provision of health services to the refugee population. Therefore, it requires immediate and often extraordinary actions to protect the health of Ukrainian refugees not only in the event of urgent illness, but also long-term medical care.

Editor-in-chief of EJTCM
Prof. Dariusz Kozłowski MD, PhD

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Protocol for a scoping review of fecal microbiota transplantation from patients into animals

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Abstract

Microbiota studies have uncovered numerous associations between human gut microbes and health-related outcomes. However, since most of these correlations were observed in cross-sectional studies, the causal inference is limited. The causal contribution of microbiota can be evidenced through disease induction or exacerbation in animal models after fecal microbiota transplantation (FMT) from patients.

In this article we present a protocol for a scoping review on the subject of FMT from humans to animals. Besides assessing how the published studies were conducted, in that scoping review we aim to find out whether enough literature exists to conduct a systematic review of the evidence for microbiota participation in the pathophysiology of any human non-infectious disease or phenotypic trait. We will conduct searches on the Web of Science platform and databases: MEDLINE, Scopus, EMBASE. Citation chasing of included studies will be done. We will include studies assessing the effects of FMT collected from people with certain medical conditions on animals. Studies that recruited only healthy humans or used other animals as donors will be excluded. The results of this literature search will be tabulated and discussed. Moreover, we will provide a short list of human non-infectious diseases or traits with the highest number of FMT patient-to-animal studies.

Keywords: Etiology · human microbiota-associated animal model · human stool transfer

Citation

Ruszkowski J, Kachlik Z, Walaszek M, Storman D, Dębska-Ślizień A. Protocol for a scoping review of fecal microbiota transplantation from patients into animals. Eur J Transl Clin Med. 2023;6(1):87-91.

DOI: [10.31373/ejtcmm/161737](https://doi.org/10.31373/ejtcmm/161737)

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Available online: www.ejtcmm.gumed.edu.pl

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Introduction

In the past decade, there has been an undeniable increase in the investigation of the structure and function of the human gut microbiota, the complex community of microorganisms that inhabit the intestinal tract. Microbiota studies have uncovered numerous associations between human gut microorganisms and both surrogate and hard outcomes. They are believed to result from dysbiosis that can be understood as a bloom of pathobionts, loss of either commensals or keystone taxa, shifts in the metabolic capacity of microbiota or loss of microbial diversity [1]. However, since the majority of these correlations were noted in cross-sectional studies comparing healthy people with those who were ill, one cannot reliably distinguish whether observed dysbiosis is a cause, consequence or an epiphenomenon of the disease [2-3].

It is believed that the answer to this question is achievable in observational studies through the adaptation of more sophisticated computational methods, commonly with longitudinal study design. However, a recent review of current statistical methods supported with a simulation study concluded that they may yield biased or misleading results due to inherent features of the microbiome data, which are zero-inflated, over-dispersed, high-dimensional, multi-collinear, multivariate and highly variable [4]. Others assess causality using data from microbiome genome-wide association studies (mGWAS) and utilize a bidirectional Mendelian randomization approach [5]. Unfortunately, the certainty of this evidence is also limited, this time because of the strong assumptions underlying Mendelian randomization [6-7]. If these assumptions are not met (e.g. due to genetic pleiotropy), the Mendelian randomization results might be not invalid. Direct evidence of a causal relationship can be obtained using experimental methods, mainly using animal models.

One such method is fecal microbiota transplantation (FMT) in which a unique microbial enterotype from healthy donors is transferred to prevent or treat diseases [8]. Since several randomized clinical trials confirmed the efficiency of FMT in the treatment of recurrent *Clostridioides difficile* infection (rCDI) [9], the causal contribution of dysbiosis to the pathophysiology of this infection has been proven. It is interpreted that FMT restores a healthy, diverse gut microbiota that protects against further episodes of rCDI. On the other hand, FMT can be used to transfer material from patients or animal models with a particular disease in order to pre-clinically induce/exacerbate it in gut dysbiosis-related disease animal models. Animal recipients (usually germ-free) of human-donated FMT are called human microbiota-associated (HMA) animal models [10-11]. Beyond proving dysbiotic microbiota contribution to a particular pathology, it is also possible, at least in some cases, to find specific pathobionts or protective microbes causally contributing to it [12].

Even though HMA animal models also have several limitations (discussed in [10-13]), they are considered currently the best model to both demonstrate causation and to elucidate mechanisms linking microbiota to the pathophysiology of human diseases. Unfortunately, there are no summaries of FMT-co-transplanted phenotypes from patients into animals nor guidelines on how to perform such studies. Specifically, no current systematic or scoping reviews on the topic were found in MEDLINE (via PubMed) or *JB I Evidence Synthesis*. Without systematic identification of studies that performed FMT from patients to create HMA animal models, it is not only impossible to reliably assess evidence supporting gut dysbiosis participation in human diseases, but also it can lead to unnecessary repetition of research along with the unethical waste of animal lives.

Currently, only a similar issue is covered by the guidelines for reporting on animal-to-animal fecal transplantation (GRAFT) studies that are based on the systematic review of murine transplantation protocols (mice were both the donor and the recipient) [14]. Even though Walter et al. systematically searched for studies reporting human microbiota-associated (HMA) rodents, they focused on the methodological and analytical limitations of such studies rather than the scope of the research [10].

We attempt to overcome the limitations of the aforementioned studies by performing a scoping review of primary studies that will answer somewhat different questions (listed below) with a broader search strategy. This scoping review's goal will be to evaluate the breadth, methodology and characteristics of the literature on FMT from humans into animals. We hope that our findings will help provide a foundation for guidelines for conducting and reporting HMA animal studies, as well as identify HMA animal models with an adequate amount of literature to conduct a systematic review with meta-analysis.

Specific research questions

1. Which human non-infectious diseases (or traits) were tried to be transplanted with FMT into animal models?
 - For each disease/trait: Which outcomes were reported?
 - For each outcome group: Did the authors report that FMT affect it?
2. How were these FMTs performed?
 - What were donor characteristics and how was the material obtained?
 - How FMT was prepared and administered?
 - What was the control for FMT?
 - What were animal characteristics, preparation and housing conditions?
3. Is there sufficient literature to conduct a systematic review with the aim of evaluating the evidence for microbiota participation in the pathophysiology of any human non-infectious disease (or phenotypic trait)?

Eligibility criteria

FMT recipients

All animal species will be considered eligible FMT recipients. These species include, but are not limited to, laboratory animal species commonly used in biomedical research (e.g. mice, rats). We will exclude studies that recruit humans to receive FMT, as it is not within the scope of our review.

Intervention

Eligible interventions include FMTs via any route (e.g. oral, rectal), frequency, and method of administration. We will include only studies that collected the material used for FMT preparation from people with certain medical conditions (e.g. colorectal cancer, hypertension) or traits (e.g. lack of response to immunotherapy). Studies that recruited only apparently healthy humans or used other animals as donors will not be eligible for the scoping review.

Control

All types of controls (e.g. vehicle solution, heat-killed FMT, FMT from healthy donor group), as well as not using control intervention, will be eligible.

Outcomes

Since we are interested in the scope of the literature, all outcomes reflecting changes in the health of FMT recipients (i.e. animals receiving FMT) will be eligible. These outcomes include, but are not limited to, the following groups: behavioral (e.g. distance traveled in open field testing), cardiovascular (e.g. left ventricular ejection fraction) and immunological (e.g. serum interleukin 17 level). Studies reporting uptake of donor microbiome profile as the only outcome of the FMT recipients (e.g. stability of human microbiome in the animal gastrointestinal tract) will be excluded because they will not answer our research questions.

Sources

This scoping review will consider both experimental and quasi-experimental study designs including randomized controlled trials, non-randomized controlled trials, before and after studies and interrupted time-series studies. We do not plan to include and analyze secondary research (e.g. systematic or narrative reviews).

Material and methods

The proposed scoping review will be conducted in accordance with the JBI methodology for scoping reviews [15-16].

Search strategy

An initial set of articles on the topic was retrieved from the study conducted by Walter et al. [10]. A full search strategy for the MEDLINE on the Ovid platform (see [Supplementary materials, Table S1](#)) was developed based on the following elements: (1) the text words contained in the titles and abstracts of relevant articles, (2) synonyms found in the MeSH thesaurus and during manual searches, (3) FMT synonyms identified by Green et al. [17], (4) a laboratory animals search filter developed by van der Mierden et al. [18]. For each included database or platform the search strategy, including all the chosen keywords and index terms, will be adjusted.

Studies published in any language will be included. The databases will be searched from the beginning until the present day.

The databases to be searched include MEDLINE (via Ovid), Scopus, EMBASE and the Web of Science platform. An additional search of the grey literature will be limited to the Open Dissertations database (via EBSCO). CitationChaser will be used to perform both backward and forward citation chasing of included studies [19].

Study selection

Following the search, all identified records will be collated and deduplicated. Following a pilot test, two independent reviewers will screen the titles and abstracts to exclude clearly irrelevant articles using the Rayyan QCRI reference manager web application [20]. Researchers will be blinded to each other's decisions. Then, all chosen potentially eligible articles will be downloaded in full-text versions and thoroughly evaluated by two reviewers using the inclusion/exclusion criteria. We will record the reason for excluding each full-text record. Because we are interested in the details of methodologies and the scope of the available evidence, conference abstracts will be classified as "awaiting assessment" studies. Any disagreements that arise between the reviewers at each stage of the selection process will be resolved after unblinding through discussion or by an additional experienced reviewer (JR or DS). The results of the search and the study selection process will be illustrated using a PRISMA 2020 flow diagram and described in the final scoping review [21].

Data Extraction

Two reviewers will independently extract data from papers included in the scoping review using a self-developed data extraction tool. We will extract specific details about the animals (e.g. species, strain, age), their preparation and housing (e.g. antibiotic/laxative pre-treatment, number of animals in cage), donor characteristics (e.g. sex, disease/trait explored), FMT preparation and storage, FMT dose and administration and the outcomes assessed. A draft extraction form is provided

(see [Supplementary Table S2](#)). As data is extracted from each included article, the draft data extraction tool will be adjusted as necessary. Modifications will be described in the scoping review. Any disagreements that arise between the reviewers will be resolved through discussion or with the help of an additional reviewer. In the case of studies awaiting assessment, we will extract the following information: (1) disease/phenotypic trait of FMT donors, (2) species and strains of FMT recipients.

Data Analysis and Presentation

Extracted human non-infectious diseases/traits that were tried to be transplanted with FMT into animal models will be clustered according to the main affected system of the human body (cardiovascular, digestive, endocrine system and metabolic diseases, integumentary, immune and lymphatic, musculoskeletal, nervous, reproductive, respiratory, urinary). We will perform clustering of the assessed outcomes during data extraction (see [Supplementary Table S2](#), rows from “5. Assessed outcomes” for proposed clusters). According to the scoping review methodology [15], the analysis of the extracted data will be limited to the basic descriptive analysis (e.g. frequency counts of animal models). Basic characteristics of studies “awaiting assessment” will be presented in a table and they will not be included in the formal statistical analyses.

To answer the first research question (“Which human non-infectious diseases or traits were tried to be transplanted with FMT into animal models?”) and its sub-questions, we plan to tabulate the data on diseases/traits as rows (grouped by the system of the human body) and outcomes clusters as columns. Additionally, the basic descriptive analysis will be narratively presented.

To answer the second research question (“How were these FMTs performed?”) and its sub-questions, we plan to present descriptive statistics in a table and discuss them in the main body of the manuscript.

To answer the third research question (“Is there sufficient literature to conduct a systematic review with the aim of evaluating the evidence for microbiota participation in the pathophysiology of any human non-infectious disease (or phenotypic trait)?”), we will provide a short list of human non-infectious diseases or traits with the highest number of FMT patient-to-animal studies. With this list, researchers will be able to select topics of interest with the assurance of finding an adequate number of relevant studies for inclusion in future systematic reviews. We will mention all the issues that can be systematically reviewed while avoiding “empty” reviews (HMA animal models with at least one study) [22].

Acknowledgements

We would like to acknowledge JBI Scoping Review Network for their extensive work on the scoping review methodology and for sharing a template for scoping review protocols.

Funding

None.

Conflicts of interest

There is no conflict of interest in this project.

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